medical
Wahr

The Journal of Laboratory and Clinical Medicine

VOL. 23 OCTOBER, 1937 No. 1

CLINICAL AND EXPERIMENTAL

COMPARATIVE EFFECTS OF THE NEW INSULIN PREPARATIONS UPON THE BLOOD SUGAR CURVE*

RESULTS WITH PROTAMINE, PROTAMINE ZINC, PROTAMINE CALCIUM, "CRYSTALLINE" AND REGULAR INSULIN

GORDON B. MYERS, M.D., AND FRANK S. PERKIN, M.D., DETROIT, MICH.

THE action of commercial insulin is so brief that three to four doses are necessary to control properly severe cases of diabetes mellitus. Attempts to prolong insulin action and more closely approximate its physiologic effect have attained varying degrees of success with the introduction of the following products:† (a) Protamine insulin; (b) zinc insulin; (c) "crystalline" insulin; (d) protamine zinc insulin; (e) protamine calcium insulin.

Since the original work of the Hagedorn group,[3] a number of papers have appeared confirming the protracted action of protamine insulin.[1, 4, 5, 7, 11, 12, 14] Scott and Fisher[13] have shown that the addition of relatively large amounts of zinc to regular insulin (1 mg. or more per 500 units) distinctly prolongs the hypoglycemic effect. Rabinowitch, Foster, Fowler and Corcoran,[10] Freund and Adler[2] and Mains and McMullen[9] have reported favorably on a "crystalline" insulin developed in the laboratories of Frederick Stearns and Company. The manufacturers state that their product crystallizes out in the test tube when

*From the Detroit Receiving Hospital and the Department of Medicine of Wayne University.

Received for publication, February 25, 1937.

Presented at the Ninth Annual Meeting of the Central Society for Clinical Research, November 6, 1936.

†The protamine, zinc, protamine zinc and protamine calcium insulin used in this work was supplied through the courtesy of Eli Lilly and Company, whereas the "crystalline" insulin was donated by Frederick Stearns and Company.

the pH is raised above 6.4 and infer that crystallization likewise occurs in the tissues, accounting for the sustained hypoglycemic effects. Rabinowitch and his coworkers have analyzed the product quantitatively for zinc by a spectrographic method, which carries an error of approximately 10 per cent, and found that it contains an average of 0.73 mg. of zinc per 500 units. By adding 1 mg. of zinc to every 500 units of commercial insulin, they were able to duplicate the clinical effects of "crystalline" insulin and, consequently, ascribed the protracted action of the latter to its zinc content. The same group also used protamine zinc insulin and found it much superior to any of the other preparations. Wilder[15] and McCullagh[8] also concluded that protamine zinc insulin had a more prolonged effect than any of the other preparations.

COMPARATIVE EFFECTS OF PROTAMINE, "CRYSTALLINE" AND REGULAR INSULIN UPON THE BLOOD SUGAR CURVE

Method.—Cooperative diabetic patients were selected for study and were placed upon a uniform weighed diet in the hospital with adequate precautions against cheating. The available glucose in the diet was divided equally among the three meals which were served at 8:00 A.M., noon, and 5:00 P.M. After a preliminary period on regular insulin, the patient was started on protamine insulin, given in two injections, the first at 7:40 A.M., and the second at 6:00 P.M. Four fractional urine specimens were collected daily and were analyzed quantitatively for glucose. On the third or fourth day after the protamine compound had been started, venous blood samples were taken at 7:00 A.M. and three hours after each meal (i.e. at 11:00 A.M., 3:00 P.M., and 8:00 P.M.) and were analyzed for glucose by the Folin-Wu method. The blood sugar should be below 0.120 per cent at these hours in properly controlled diabetics. In the first few cases, the blood sugar determinations were repeated on the seventh or tenth day, but it was found that this curve closely resembled the first one taken on the third or fourth day, as illustrated in Table I. In the later cases the second curve was, therefore, dispensed with. "Crystalline" and regular insulin were then substituted in rotation for protamine insulin, using exactly the same dosage, given at the same hours, with blood determinations at 7:00 A.M., 11:00 A.M., 3:00 P.M., and 8:00 P.M. on the third or fourth day. Some patients were then placed upon regular or "crystalline" insulin at 7:40 A.M. and protamine insulin at 9:00 P.M. in the same dosages and the blood sugar curve was again checked.

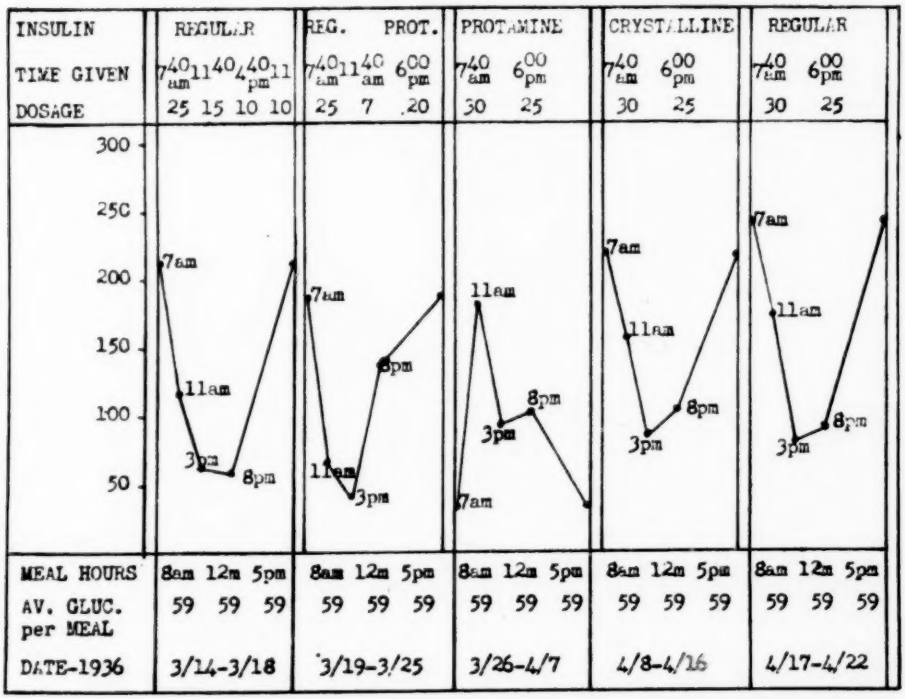
TABLE I

COMPARISON OF CONSECUTIVE CURVES ON A CONSTANT DOSE OF PROTAMINE INSULIN

	AVE. NO. OF DAYS ON PROTAMINE INSULIN	AVE. BLOOD SUGAR IN 13 CASES			
		7 A.M.	11 A.M.	3 P.M.	8 P.M.
First curve	3.2	0.097%	0.179%	0.142%	0.143%
Second curve	7.7	0.085	0.187	0.155	0.154

Results in Individual Cases.—The blood sugar curves obtained with uniform doses of protamine, "crystalline" and regular insulin have been charted for purposes of comparison.*

CASE R-3600 (Chart 1).—A forty-eight-year-old male was started upon 60 units of regular insulin daily, divided into four doses, the last of which was given at 11:00 P.M. In spite of this dose, he had a fasting blood sugar above 0.200 per cent. When a single dose of protamine insulin at 6:00 P.M. was substituted for the last two doses of regular insulin, the most notable difference lay in the tendency toward severe insulin reactions in the mid-afternoon. As a general rule, the blood sugar was better stabilized and hypoglycemic reactions were



EFFECT OF THE DIFFERENT FORMS OF INSULIN UPON THE BLOOD SUGAR CURVE

Chart 1

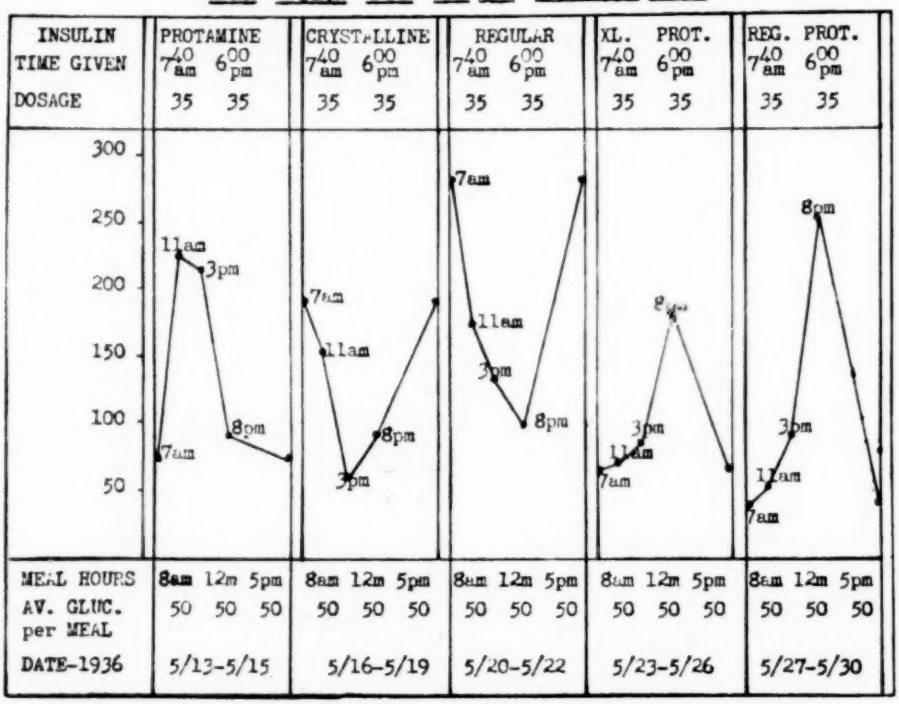
less frequent and severe when protamine insulin was used exclusively than when regular insulin was given in the morning and protamine in the evening. The last three curves represent averages of two or more determinations made while the patient was on constant doses of protamine, "crystalline" and regular insulin, respectively. The curves on "crystalline" and regular insulin are strikingly similar to one another but just the opposite of the curve on protamine insulin. The high fasting levels in the former curves would indicate that the effect of regular and "crystalline" insulin lasted less than thirteen hours. On the other hand, hypoglycemic levels were regularly observed thirteen or more hours after protamine insulin, often without symptoms of an insulin reaction. The retardation in protamine insulin action is

*To complete the twenty-four-hour curve, a line was drawn from the 8:00 P.M. reading back to the 7:00 A.M. starting level. The urinalyses were omitted from the chart since they yielded no information that could not be derived from the blood sugar curves.

evident in the hyperglycemia at 11:00 A.M. A morning rise in blood sugar could be avoided, in some cases, by giving the protamine insulin an hour or more before breakfast.

CASE R-5646 (Chart 2).—A forty-two-year-old male was admitted to the hospital with a simple fracture of the femur. During the period depicted in the chart, he was on a uniform diet and a constant insulin dosage. The differences between the curves on protamine insulin and the curves on "crystalline" or regular insulin which were noted in the preceding case were also present here. When the patient was given "crystalline" or regular insulin in the morning and protamine at 6:00 P.M. the prolonged hypoglycemic effect of the latter was demonstrable in the low fasting level, whereas the more rapid action of the former kept the blood sugar down during the day. The hyperglycemia at 8:00 P.M. suggests that the effect of the morning dose of "crystalline" or regular insulin had worn off, while that of the 6:00 P.M. dose of protamine had scarcely begun.

CASE R-5646 MALE AGE 42 FRACTURED FEMUR



EFFECT OF THE DIFFERENT FORMS OF INSULIN UPON THE BLOOD SUGAR CURVE

Chart 2

Summary of Results.—The comparative effects of protamine, "crystalline" and regular insulin upon the blood sugar curve were determined in ten cases in the manner described above. In seven of these, all relatively insulin sensitive, the results were similar to those illustrated in Charts 1 and 2. In three cases, each relatively insulin resistant, rather uniform plateau curves were obtained with all three types of insulin. One of these patients had an acute salpingitis; the other two were in the sixth decade and had moderate arteriosclerosis but no other demonstrable complications.

The average curves with each type of insulin in the ten cases are given in Table II. A striking similarity in the curves on "crystalline" and regular insulin is evident in the table and would seem to indicate that there is little, if

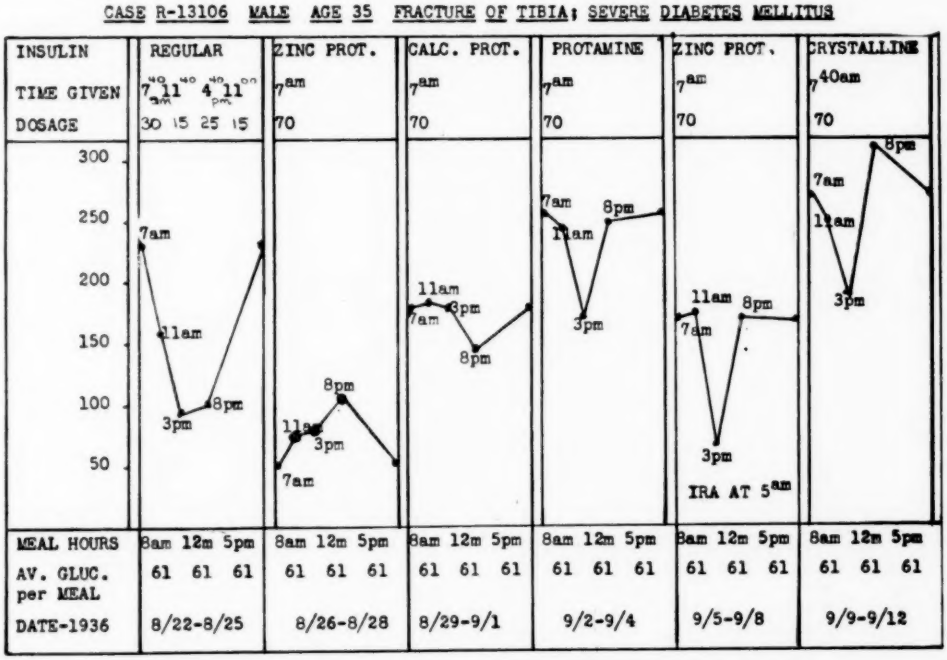
any, difference in the action of "crystalline" and regular insulin when used in the manner prescribed for these patients. The normal or hypoglycemic fasting levels regularly observed when protamine insulin was given at 7:40 A.M. and

TABLE II

AVERAGE BLOOD SUGAR CURVES IN 10 CASES ON PROTAMINE, "CRYSTALLINE" AND REGULAR INSULIN

TYPE OF INSULIN	AVE. DOSAGE	AVE. OF 11 DETERMINATIONS			
		7 A.M.	11 A.M.	3 P.M.	8 P.M.
Regular	53-U	0.195%	0.161%	0.133%	0.118%
"Crystalline"	53	0.195	0.176	0.127	0.142
Protamine	53	0.082	0.177	0.157	0.139

6:00 P.M. would indicate that its action exceeded thirteen hours. When the last dose of "crystalline" insulin was given at 6:00 P.M., the fasting blood sugar was



EFFECT OF THE DIFFERENT FORMS OF INSULIN UPON THE BLOOD SUGAR CURVE

Chart 3

elevated but when the last dose was given at 9:00 P.M., the fasting blood sugar was normal. This would suggest that the effect of "crystalline" insulin lasts between ten and thirteen hours. The fall in blood sugar between 7:00 A.M. and 11:00 A.M. when regular or "crystalline" insulin was given at 7:40 A.M., in contrast to the rise at this time when protamine was used, illustrates the more prompt action of the former and the retarded effect of the latter.

COMPARATIVE EFFECTS OF PROTAMINE ZINC, PROTAMINE CALCIUM AND PLAIN PROTAMINE INSULIN ON THE BLOOD SUGAR CURVE

Method.—The procedure was similar to that employed above in the comparison of protamine, "crystalline" and regular insulin except that the protamine compounds were given in a single injection daily. Preliminary trials were made

on five patients to determine the optimum time for a single dose. These patients could be satisfactorily controlled when the dose was given either at 7:00 A.M. (one hour before breakfast) or 11:00 P.M., but the former hour was preferable. Doses at 6:00 P.M. were much less efficacious. It was consequently decided to give the preparations once daily at 7:00 A.M.

Results in Individual Cases.—

CASE R 13106 (Chart 3).—A thirty-five-year-old male was admitted to the hospital with a simple fracture of the tibia and was placed upon 85 units of commercial insulin daily, the last dose given at 11:00 P.M. In spite of this, his fasting blood sugar was above 0.200 per cent. A single injection of 70 units of protamine zinc insulin at 7:00 A.M. daily was then substituted for the regular insulin and the blood sugar on the third day ranged between 0.050 per cent and 0.100 per cent. Plateau curves at considerably higher levels were obtained with protamine calcium and plain protamine insulin. The patient was again placed on protamine zinc insulin but the curve was modified by a severe reaction which occurred at 5:00 A.M. on the day when the blood sugar determinations were made. The reactions from protamine zinc insulin were more severe than those from plain protamine and were prone to occur in the early morning (seventeen to twenty-four hours after the injection was given). Such reactions have been avoided in outpatients by a small bedtime feeding. The results obtained with "crystalline" insulin were inferior to those with any of the protamine preparations. On the other hand, surprisingly good results were obtained with a single dose of commercial insulin containing 1 mg. of zinc per 500 units.

Summary of Results.—The comparative effects of protamine zinc, protamine calcium and plain protamine insulin upon the blood sugar curve were determined in ten severe diabetics, who took a uniform dose in a single injection at 7:00 A.M. daily. The average of fourteen series of curves with each type of insulin is given in Table III. The superiority of protamine zinc insulin is

TABLE III

AVERAGE BLOOD SUGAR CURVES IN 10 CASES
PLAIN PROTAMINE, PROTAMINE CALCIUM AND PROTAMINE ZINC INSULIN

TYPE OF INSULIN	AVE. DOSAGE	AVE. OF 14 DETERMINATIONS			
		7 A.M.	11 A.M.	3 P.M.	8 P.M.
Protamine	46-U	0.143%	0.242%	0.166%	0.163%
Protamine calcium	46	0.146	0.206	0.149	0.098
Protamine zinc	46	0.069	0.125	0.131	0.112

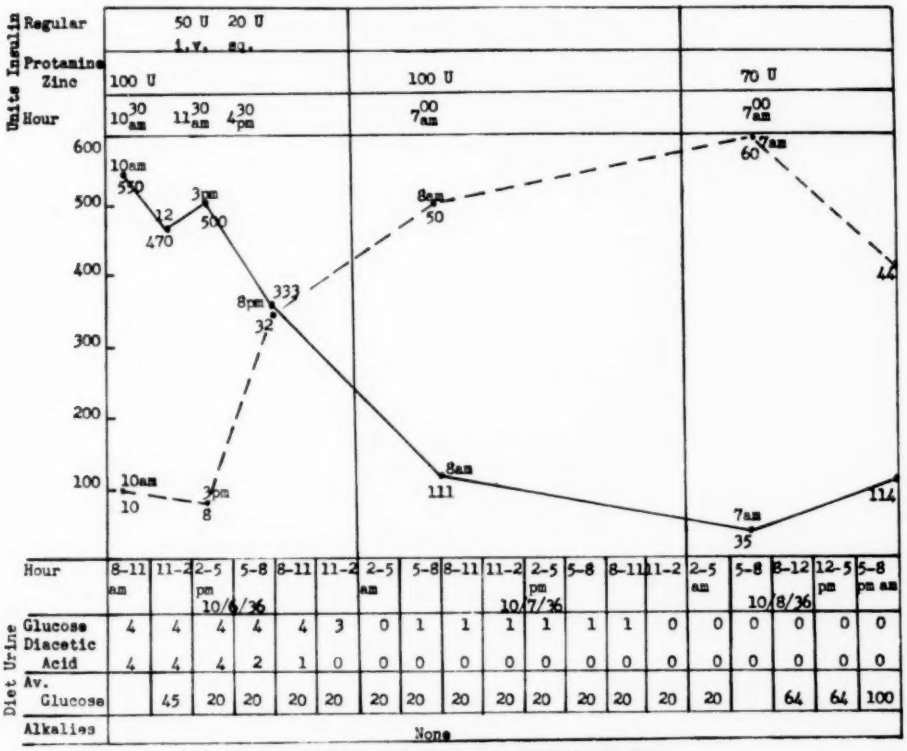
evident from a study of the table. Since the average 7:00 A.M. blood sugar was 0.069 per cent, the action of protamine zinc insulin apparently exceeds twenty-four hours. The maximal effect of protamine calcium insulin was noted in the 8:00 P.M. specimen taken thirteen hours after the injection. The rise in blood sugar during the night would indicate that the hypoglycemic effect of protamine calcium insulin does not last twenty-four hours. The blood sugar on equivalent doses of plain protamine insulin was constantly above the limits of normal.

COMMENT

The immediate results with protamine zinc insulin were much superior to those with any of the other preparations. Nineteen patients have been carried on a single dose of protamine zinc insulin for a period of three to six months.

The insulin requirement has fallen in several and has not increased in any. None has shown clinical evidence of zinc poisoning.

Advantages and Disadvantages of Protamine Zinc Insulin, as compared with regular insulin: (1) A drastic reduction in the number of injections daily and a moderate reduction in total dosage is possible. A group of 34 patients was better controlled on a single dose of protamine zinc insulin averaging 37 units than on an average of 3.0 doses of regular insulin daily, amounting to 51 units. The fasting levels were lower and the blood sugars were better stabilized throughout the day with protamine zinc insulin. (2) Hypoglycemic reactions



USE OF PROTAMINE ZINC INSULINATE IN DIABETIC COMA
Solid line = blood sugar; broken line = blood CO_2 combining power

Chart 4

were less frequent with protamine zinc insulin, but more protracted and severe. When the injection was given before breakfast, reactions were prone to occur during sleeping hours. Glucose orally or intravenously was necessary to control the immediate symptoms, supplemented by starch to prevent relapse into hypoglycemia. Such reactions may usually be prevented by a bedtime feeding. Nineteen patients were well controlled without reactions by distributing the available glucose as follows: One-sixth of the total at breakfast, one-third at dinner, one-third at supper, and one-sixth at bedtime.

Use of Protamine Zinc Insulin in Diabetic Coma.—Protamine zinc insulin was used successfully as a supplement to regular insulin in several cases of

diabetic coma. The results will be reported in detail in a separate communication. They are exemplified by the following case:

CASE R-17053 (Chart 4).—A colored female, aged twenty-six years, was admitted at 10:00 A.M. on Oct. 6, 1936 in a typical diabetic coma with a blood sugar of 0.550 per cent and carbon dioxide combining power of 10 volumes per cent. She received 100 units of protamine zinc insulin at 10:30 A.M., 50 units of regular insulin intravenously at 11:30 A.M. and 20 units subcutaneously at 4:30 P.M. There was little change until late afternoon when the blood sugar commenced to fall and the blood carbon dioxide-combining power began to rise, reaching 0.333 per cent and 32 volumes per cent, respectively, by 8:00 P.M. and 0.111 per cent and 50 volumes per cent, respectively, by eight o'clock the next morning. The change between 8:00 P.M. and 8:00 A.M. was probably due largely to the protamine zinc insulin. This patient had been admitted two months previously in diabetic coma with an initial blood carbon dioxide combining power of eight volumes per cent. On that occasion regular insulin was used exclusively. Twenty doses, totalling 300 units, were required to bring the patient out to a similar degree as was attained on her second admission by two doses, totalling 70 units, supplemented by 100 units of protamine zinc insulin.

SUMMARY

1. The comparative effects of uniform doses of protamine, "crystalline" and regular insulin at 7:40 A.M. and 6:00 P.M. upon the blood sugar before breakfast and three hours after each meal were studied in a group of ten diabetic patients. From the parallelism in the curves on regular and "crystalline" insulin, it would appear that there is little, if any, difference in the action of these two preparations when used in the manner prescribed for these patients. The duration of the hypoglycemic effect of protamine insulin was considerably greater than that of "crystalline" or regular insulin.

2. The comparative effects of single doses of protamine zinc, protamine calcium and plain protamine insulin at 7:00 A.M. daily were studied in ten severe diabetic patients. The results with protamine zinc insulin were much superior to those with any of the other preparations.

3. A group of 34 patients was better controlled on a single dose of protamine zinc insulin averaging 37 units than on an average of 3.0 doses of regular insulin daily, amounting to 51 units.

4. Severe reactions were prone to occur between midnight and 7:00 A.M., seventeen to twenty-four hours after the administration of protamine zinc insulin. They can be prevented by a bedtime feeding.

5. Protamine zinc insulin was used successfully as a supplement to regular insulin in the treatment of diabetic coma.

The patients used in this study were on the Metabolic Service of Dr. Richard M. McKean. We are indebted to Mrs. Virginia Weeks for valuable technical assistance.

REFERENCES

1. Campbell, W. R., Fletcher, A. A., and Kerr, R. B.: Protamine Insulin in the Treatment of Diabetes Mellitus, Am. J. M. Sc. **192:** 589, 1936.
2. Freund, H. A., and Adler, S.: Effects of Standard, Protamine and Crystalline Insulin on Blood Sugar Levels, J. A. M. A. **107:** 573, 1936.
3. Hagedorn, H. C., Jensen, B. N., Krarup, N. B., and Wodstrup, I.: Protamine Insulinate, J. A. M. A. **106:** 177, 1936.
4. Joslin, E. P.: The Old and New Insulin in Diabetes, Canad. M. A. J. **35:** 526, 1936.
5. Kerr, R. B., Best, C. H., Campbell, W. R., and Fletcher, A. A.: Protamine Insulin, Canad. M. A. J. **34:** 400, 1936.

6. Krarup, N. B.: Clinical Investigations Into the Action of Protamine Insulinate, G. E. C. Gad., Copenhagen, 1935.
7. Lawrence, R. D., and Archer, N.: Some Experiments With Protamine Insulinate, Brit. M. J. **1**: 747, 1936.
8. McCullagh, E. P.: Protamine Zinc Insulin in Diabetes, Cleveland Clin. Quart. **4**: 26, 1937.
9. Mains, M. P., and McMullen, C. J.: Clinical Investigation of Improved Crystalline Insulin; Preliminary Report, J. A. M. A. **107**: 959, 1936.
10. Rabinowitch, I. M., Foster, J. S., Fowler, A. F., and Corcoran, A. C.: Clinical Experiences With Protamine Zinc Insulin and Other Mixtures of Zinc and Insulin in Diabetes Mellitus, Canad. M. A. J. **35**: 239, 1936.
11. Richardson, R., and Bowie, M. A.: Observations in the Effectiveness of Protamine Insulin, Am. J. M. Sc. **192**: 764, 1936.
12. Root, H. F., White, Priscilla, Marble, A., and Stotz, E. H.: Clinical Experience With Protamine Insulinate, J. A. M. A. **106**: 180, 1936.
13. Scott, D. A., and Fisher, A. M.: Effect of Zinc Salts on Action of Insulin, J. Pharmacol. & Exper. Therap. **55**: 206, 1935.
14. Sprague, R. G., Blum, B. B., Osterberg, A. E., Kepler, E. J., and Wilder, R. M.: Clinical Observations With Insulin Protamine Compound, J. A. M. A. **106**: 1701, 1936.
15. Wilder, R. M.: The New Insulin, Minn. Med. **20**: 6, 1937.

GUANIDINE-LIKE SUBSTANCES IN THE BLOOD*

III. Blood Guanidine in Normal Pregnancy, Toxemias of Pregnancy, and Cirrhosis of the Liver

Jerome E. Andes, Ph.D., M.D., Erlene J. Andes, M.S., Morgantown, W. Va., and Victor C. Myers, Ph.D., D.Sc., Cleveland, Ohio

MINOT and Cutler,[1] in 1929, presented data which showed that the symptoms of chloroform and carbon tetrachloride poisoning closely resemble those of guanidine poisoning. They noted especially a central necrosis of the liver, and an increase in the blood guanidine concentration. They found that calcium therapy relieved the symptoms of poisoning and lowered the blood guanidine concentration. The first method of Major and Weber,[2] which does not take into account the color due to creatine was used. In 1929, the same authors[3] published the results of further work, employing Major and Weber's new method,[4] although the correction for the color given by creatine was not applied. They found normal guanidine values in carcinoma of the liver, syphilis, hepatitis, and alcoholic cirrhosis of the liver, but one case of arsphenamine jaundice and one of congenital abnormality of the liver gave increased figures. They also noted an increased blood guanidine concentration in eclampsia, especially true eclampsia.

Ellsworth,[5] in 1930, using the same method (uncorrected as above), found the blood guanidine to be normal in hemolytic jaundice, but markedly increased in Laennec's cirrhosis and arsphenamine jaundice. He further noted[6] that the injection of guanidine by mouth gave no increase in the blood guanidine of normal individuals, but caused a definite and lasting increase in patients with cirrhosis of the liver. Malméjac and coworkers[7] and also Malméjac[8] found the

*From the Departments of Biochemistry and Obstetrics, Western Reserve University, School of Medicine, Cleveland, Ohio.
Received for publication, February 3, 1937.

blood guanidine increases during normal pregnancy, especially near term. They found this condition more pronounced in eclampsia and preeclampsia, depending on the severity of the condition, and that recovery is accompanied by a decrease in the concentration of blood guanidine. Malméjac[9] also found a hypocalcemia paralleling the guanidinemia, and strongly urged calcium therapy. He believes that normal childbirth shows a hepatoparathyroid dyssynergia, and that eclampsia and related diseases are merely an increased severity of the normal condition.

Cutler,[10] in 1931, using the same method but correcting for the color given by creatine, found the acute liver injury in chloroform, carbon tetrachloride, arsenic, and phosphorus poisoning was accompanied by an increase in the blood guanidine. Although her patients exhibited some tendency to nitrogen retention, she apparently believed that the hyperguanidinemia was due to liver injury. Arkawa,[11] the next year, reported an increase in blood guanidine in proportion to the severity of the hepatic damage; later Minot and Dodd[12] added additional evidence along this line and suggested calcium therapy in all such cases. Zappacosta[13] in 1935 noted that the removal from the blood stream of orally administered guanidine is diminished in diseases with impaired liver function, and recommended this procedure as a test of the degree of impairment of hepatic function.

A number of dissenting notes are found to this array of evidence (in favor of the relationship between liver disease and guanidine). Remond and Colombies,[14] in 1930, found no relation between the level of blood guanidine and the degree of liver function. Hepler and Simmonds,[15] the next year, produced liver injury by ligating the hepatic veins, and did not find an increase in the guanidine concentration of the blood. In 1932, de Wesselow and Griffith[16] observed normal or low values in preeclampsia and eclampsia; also Turries[17] claimed to have found low guanidine concentrations in the blood of patients with severe hepatic disease. The same year Stander[18] published 12 determinations on patients with eclampsia, which showed blood guanidine values within the same range as given by his normals. However, he only gave three normal determinations for comparison, and did not apply the correction for the color given by creatine. After injecting guanidine into rabbits, he found an increase in both guanidine and nonprotein nitrogen concentrations in the blood, but found no evidence of central necrosis of the liver. Finally Krieger,[19] in 1934, found the blood guanidine normal in all his cases of eclampsia.

EXPERIMENTAL

Blood Guanidine During Normal Pregnancy.—In the study of blood guanidine presented in this paper, we employed the method described in Paper I of this series.[20] In Table V of this same paper is given the results of blood guanidine determinations on 17 normal females in the early part of gestation. The average value is 0.23 mg. per 100 c.c. of whole blood, with a range of 0.18 to 0.28 mg. Additional data are presented in Table I of this paper on 8 normal women just a few hours before delivery. The results of this study must be regarded as entirely normal, the average guanidine value being slightly below that shown earlier in gestation. In Table II are recorded the guanidine determinations on the blood of the *same* eight women from three

to five days after delivery. The guanidine concentration was found to be elevated in every case, the average value being almost twice that observed before delivery (0.38 mg. after delivery as compared to 0.20 mg. before). In determinations not recorded here, it was found that the hyperguanidinemia after delivery begins soon after parturition, increases to a maximum during the third to seventh days, and then decreases to practically normal within three weeks.

TABLE I

BLOOD GUANIDINE OF NORMAL PREPARTUM MATERNITY PATIENTS

(Figures in mg. per 100 c.c.)

CASE	AGE	BLOOD PRESSURE	UREA NITROGEN	BLOOD SUGAR	BLOOD GUANIDINE
1	25	120/80	7.5	86	0.20
2	18	110/90	10.2	89	0.20
3	40	118/68	8.9	82	0.17
4	25	110/68	10.7	102	0.19
5	25	124/86	8.6	95	0.23
6	21	119/77	8.9	84	0.22
7	26	110/75	7.9	72	0.22
8	28	115/82	9.7	89	0.21
		Average			0.20

TABLE II

BLOOD GUANIDINE OF NORMAL POSTPARTUM MATERNITY PATIENTS

(Figures in mg. per 100 c.c.)

CASE	AGE	DAYS POST-PARTUM	BLOOD PRESSURE	UREA NITROGEN	BLOOD SUGAR	BLOOD GUANIDINE
1	25	3	125/82	8.7	89	0.34
2	18	3	115/90	8.5	94	0.32
3	40	4	115/70	8.3	98	0.43
4	25	4	120/78	7.8	80	0.31
5	25	5	120/90	9.8	95	0.44
6	21	4	110/70	9.4	104	0.36
7	26	"	120/82	7.3	102	0.46
8	28	3	100/70	8.1	110	0.36
			Average			0.38

A few experiments showed this increase in guanidine after delivery to be almost entirely in the cells. In Table III are shown a series of determinations of the guanidine content of the whole blood and oxalated plasma (normal in-

TABLE III

DISTRIBUTION OF GUANIDINE IN THE CELLS AND PLASMA OF NORMAL INDIVIDUALS

CASE	GUANIDINE IN 100 C.C. OF BLOOD MG.	GUANIDINE IN 100 C.C. OF PLASMA MG.	GUANIDINE IN 100 C.C. OF CELLS* MG.	RATIO OF GUANIDINE IN CELLS TO PLASMA
1	0.24	0.14	0.36	2.6
2	0.23	0.14	0.34	2.4
3	0.24	0.12	0.38	3.2
4	0.18	0.13	0.24	1.9
5	0.23	0.14	0.34	2.4
Mixed Blood	0.26	0.16	0.38	2.4
Average	0.23	0.14	0.34	2.4

*Calculated assuming the cell volume to be 45 per cent.

dividuals). The concentration in the plasma averaged about 0.14 mg. per 100 c.c., and in the cells (calculated assuming a cell volume of 45 per cent) about 0.34 mg. This shows that the concentration of guanidine in the cells is about 2.4 times that of the plasma. In Table IV are shown the results of guanidine determinations on the whole blood and oxalated plasma of five normal postpartum patients. The guanidine values found for the plasma are practically the same as those found with the normal individuals, but the cell guanidine (calculated as before) has increased to a value more than double that found in the normal cells. The average ratio of guanidine in the cells to that in the plasma has risen from 2.4 to 5.5. Calculations easily show that this change in the blood guanidine after delivery cannot be due to blood concentration.

TABLE IV

DISTRIBUTION OF GUANIDINE IN THE CELLS AND PLASMA OF NORMAL POSTPARTUM MATERNITY PATIENTS

CASE	GUANIDINE IN 100 C.C. OF BLOOD MG.	GUANIDINE IN 100 C.C. OF PLASMA MG.	GUANIDINE IN 100 C.C. OF CELLS* MG.	RATIO OF CELL TO PLASMA GUANIDINE
1	0.43	0.14	0.80	5.8
2	0.44	0.18	0.76	4.2
3	0.36	0.20	0.56	2.8
4	0.46	0.11	0.89	8.1
5	0.36	0.12	0.65	5.4
Average	0.41	0.15	0.73	5.5

*Calculated assuming the cell volume to be 45 per cent.

Blood Guanidine in Toxemias of Pregnancy.—Table V shows the results of 112 blood guanidine determinations on 37 patients diagnosed as toxemia of pregnancy, and two determinations on a case of septicemia. With some of the cases, a number of determinations were possible, extending over a period of weeks or months, but with several cases only one sample of blood could be procured. Simultaneous analyses were made for the urea, nonprotein nitrogen, creatinine, sugar, and (in some) uric acid on the same sample of blood.

An analysis of the table brings out several interesting points. First, there is a rise in the blood guanidine after delivery, which in mild cases is similar to that found in normal postpartum patients. With severe toxemias, the guanidine values are increased above those observed in the normal cases, occasionally the increase being accompanied by nitrogen retention. (See Cases 1, 5, 7, 12, and 21.) Furthermore, in eclampsia the condition of hyperguanidinemia continues as long as the toxic condition exists. Case 21 shows a blood guanidine value of 0.63 mg. twenty-two days after delivery. In preeclampsia, there is also an elevation of the blood guanidine concentration, which increase was not found in any of the normal antepartum cases studied. A pronounced hyperguanidinemia was only found in the most severe cases, and was often accompanied by a tendency for nitrogen retention (see Case 7). The mild cases showed a practically normal blood guanidine concentration. Furthermore, any marked increase in the guanidine was accompanied by very grave symptoms (either in eclampsia or preeclampsia); generally convulsions, and patients in convulsions always showed markedly increased blood guanidine values (see

TABLE V

BLOOD GUANIDINE IN TOXEMIAS OF PREGNANCY

Figures in mg. per 100 c.c.

PATIENT	DATE 1931	BLOOD PRESSURE MM. HG	BLOOD GUANIDINE	UREA NITROGEN	NON-PROTEIN N.	CREATININE	SUGAR	REMARKS
1	4/17	148/120	0.36	8.8	43	1.4	85	Aged 29 Para vi
	5/1	154/122	0.23	5.0	16	1.8	83	
	5/15	180/130	0.28	7.7	20	1.1	77	
	5/29	150/128	0.37	5.0	17	1.1	69	
			Delivered June 6, 1931					
	6/11	170/138	0.29	5.4	15	1.2	87	
	6/15	184/150	0.46	No Chemistry		Patient eventually discharged		
2	4/17	142/114	0.41	9.2	30	1.9	96	Aged 36 Para iii
			Delivered April 30, 1931					
3	4/10	142/144	0.38	8.8	43	1.4	85	Aged 29 Para i
			(Delivered 24 hours previously)					
4	4/17	150/120	0.36	7.1	26	1.8	85	Aged 28
	4/24	135/120	0.31	10.3	24	1.3	81	Para ii
			Delivered May 4, 1931					
	5/5	136/96	0.32	9.8	24	1.4	76	
	5/11	140/98	0.28	11.0	24	1.7	108	Dismissed
5	4/21	180/120	0.58	8.2	25	1.7	85	B.P. on admission = 210/130
	4/29	148/108	0.33	8.4	24	1.7	79	Aged 42
	5/5	198/120	0.31	[illegible]	21	1.6	91	
			Delivered May [illegible], 1931					
	5/19	140/90	0.45	8.9	30	1.5	91	Dismissed
6	5/24	115/80	0.25	7.0	27	1.9	77	Aged 25 Para ii
			Patient had delivered about 3 hours previously					
7	4/20	138/110	0.32	8.7	27	1.7	90	Aged 28 Para ii
	4/26	160/110	0.49	15.0	32	1.9	81	
	5/4	160/118	0.38	22.0	45	1.7	78	
	5/11	210/140	0.43	18.0	39	1.7	82	
	5/15	200/180	0.36	22.0	47	2.0	82	
	5/21	190/130	0.37	12.0	31	2.3	87	
	5/29	210/140	0.41	18.0	40	1.9	76	
			Delivered June 6, 1931		Condition Serious—Convulsions			
	6/13	128/92	0.73	17.0	36	1.8	84	Improved Discharged soon afterward
8	5/1	136/90	0.33	9.3	24	1.6	82	Aged 33 Para iii
			Delivered previously on April 17, 1931					
9	4/18	120/85	0.27	9.4	30	1.3	71	Aged 22 Para iii
			Delivered about 2 hours previously					
10	5/5	124/70	0.29	5.7	17	1.4	78	Aged 20 Para iii
			Delivered previously on April 18, 1931					
11	5/5	160/110	0.33				269	Aged 35 Para i
	5/7	130/70	0.28				200	Diabetes Mel.
			Left Hospital Undelivered					
12	5/29	180/138	0.25	7.0	20	1.5	75	Para iii Aged 27
	6/12	176/138	0.32	13.6	31	1.1	92	
			Delivered June 26, 1931					

TABLE V—CONT'D

PATIENT	DATE 1931 AND 1932	BLOOD PRESSURE MM. HG	BLOOD GUANIDINE	UREA NITROGEN	NON-PROTEIN N.	CREATININE	SUGAR	REMARKS
13	6/4	140/80	0.26	7.5	21	1.7	75	Aged 34 Para vi
	6/11	120/80	0.22	5.4	20	1.7	65	
			Delivered June 22, 1931					
14	6/16	180/136	0.25	6.3	17	1.5	84	Aged 19 Para v
			Delivered five hours afterward					
15	6/9	208/140	0.39	9.8	29	1.9	80	Aged 33 Para ii
	6/13	180/134	0.25	11.5	28	1.6	112	
			Delivered 24 hours later—forceps					
	6/22	160/100	0.38	6.7	18	1.7	100	Discharged
16	6/16	144/100	0.28	8.3	21	1.8	71	Aged 22 Para v
	6/23	130/80	0.24	10.2	23	1.8	73	
			Delivered 6 hours afterward—Baby dead					
17	10/14	170/120	0.31	13.8	24	1.4	80	Aged 35 Para viii
			Delivered Oct. 18, 1931					
	10/21	150/100	0.58	7.6	25	1.7	87	
	10/28	178/88	0.30	7.6	19	1.7	99	Discharged
18	9/2	140/100	0.29	15.4	25	1.5	81	No other data available
19	9/5	170/120	0.31	7.5	23	1.3	83	Aged 26 Para iii
	9/8	174/120	0.41	7.2	20	1.5	81	
			Delivered Sept. 14, 1931					
	9/21	160/120	0.42	6.3	18	1.3	101	
	9/23	140/90	0.36	8.3	29	1.2	79	Discharged
20	10/29	160/100	0.32	7.8	23	1.5	83	Aged 46 Para vi
	11/6	150/95	0.26	7.3	19	1.2	95	
			Delivered Nov. 19, 1931					
	11/20	140/98	0.32	5.0	19	1.3	83	
	11/30	122/98	0.27	7.0		1.2	123	Discharged
21	9/8	160/110	0.63	7.7	20	1.6	150	Aged 33 Para vii
			Patient delivered 23 days previously in convulsions					
22	11/8	194/124	0.28	5.6	22	1.3	101	Aged 33 Para vi
	11/13	160/110	0.23	9.0	21	1.9	67	
	11/19	172/128	0.43	7.3	15	1.6	68	
			Delivered several hours later					
	11/30	150/110	0.29	12.0	19	1.2	93	Discharged
23	11/20	140/100	0.29	7.5	25	1.6	70*	Aged 33 Para v
	12/1	126/80	0.23	7.7	23	1.5	68	
			Delivered Dec. 6, 1931					
	12/7	160/100	0.31	12.8	27	1.7	77	Discharged
24	11/23	130/80	0.27	9.7	24	1.5	50	Aged 23 Para i
			Delivered Nov. 29, 1931					
	12/1	130/80	0.34	15.4	32	1.6	67	Discharged
25	11/25	170/110	0.46	8.3	23	1.4	60	Aged 33 Para viii
	11/30	150/76	0.37	7.5	18	1.2	88	
			Delivered Dec. 31, 1931					
	1/4	125/85	0.29	6.9	27	1.4	73	
	1/6	120/80	0.38	10.7	31	1.5	71	Discharged

*The blood sugar values from this point on were determined with Benedict's latest copper method.

TABLE V—CONT'D

PATIENT	DATE 1931 AND 1932	BLOOD PRESSURE MM. HG	BLOOD GUANIDINE	UREA NITROGEN	NON-PROTEIN N.	CREATININE	SUGAR	REMARKS
26	12/10	190/140	0.30	10.5	44	1.5	44	Aged 33 Para xi
	12/18	158/120	0.26	6.6	27	1.4	72	
			Delivered Jan. 3, 1932					
	1/6	168/120	0.32	11.2	33	1.5	66	
	1/12	168/120	0.27	12.8	24	1.5	84	Discharged
27	12/7	130/90	0.33	9.0	23	1.6	71	Aged 21 Para i
	12/16	140/110	0.29	11.0	34	1.5	84	Delivered 12/6/31
28	12/29	160/130	0.26	9.1	28	1.4	52	Aged 35 Para i
	1/4	118/76	0.33	12.2	28	1.3	84	
	1/6	118/84	0.20	5.1	21	1.5	66	
			Delivered Jan. 11, 1932					
	1/19	118/72	0.28	15.0	25	1.5	117	Discharged
29	1/16	114/66	0.20	14.5	26	1.4	118	Aged 35 Para iii
			Delivered Jan. 18, 1932					
	1/19	138/78	0.19	17.4	28	1.6	82	
	1/22	134/90	0.36	13.5	25	1.5	100	Discharged
			Delivered Jan. 15, 1932					
30	1/22	110/80	0.36	14.0	24	4.2†	101	Discharged Aged 21 Para i
31	2/3	140/100	0.22	17.0	33	4.3	98	Aged 20 Para i
	2/9	146/100	0.28	14.4	28	4.7	96	
	2/13	140/100	0.21	13.8	24	5.2	96	
			Delivered Feb. 15, 1932					
	2/17	140/100	0.30	14.0	22	7.5	100	
	2/23	134/90	0.36	10.3	23	5.3	97	
	2/26	140/78	0.37	11.8	24	4.9	88	
	3/1	112/78	0.35	8.2	24	4.6	86	Discharged 2 days later
32	2/5	140/88	0.58	13.1	22	8.2	160	Aged 18 Para i
			Delivered the same day—in convulsions					
	2/9	143/112	0.31	13.5	21	7.0	133	
	2/17	110/80	0.25	15.4	25	4.3	101	Discharged
	Delivered Feb. 7, 1932			Diagnosis Septicemia				
33	2/9	100/55	0.26	17.5	30	9.6	145	Aged 26 Para i
	2/13	108/48	0.22	14.5	21	4.0	120	Died
			Delivered Feb. 14, 1932					
34	2/17	138/78	0.32	14.5	26	4.4	102	Aged 33 Para i
	2/22	132/108	0.37	12.8	24	6.0	86	Discharged
35	2/6	160/120	0.22	11.9	20	5.6	66	Aged 18 Para ii
	2/9	160/112	0.20	10.1	18	3.4	86	
	2/17	140/100	0.25	8.8	18	3.4	63	
	2/18	150/110	0.34	10.7	20	4.0	71	
	2/23	140/112	0.25	9.4	20	4.8	78	
			Delivered Feb. 25, 1932					
	2/26	154/112	0.33	10.0	21	4.7	61	
	3/1	148/98	0.38	8.2	20	4.0	100	
	3/4	128/100	0.32	8.7	21	3.9	75	Discharged

†Values from this point on refer to uric acid.

TABLE V—CONT'D

PATIENT	DATE 1932	BLOOD PRESSURE MM. HG	BLOOD GUANIDINE	UREA NITROGEN	NON-PROTEIN N.	CREATININE	SUGAR	REMARKS
			Delivered Feb. 16, 1932					
36	2/17	140/100	0.33	16.0	30	6.5	100	Aged 19 Para i
	2/23	140/118	0.32	15.0	32	5.6	83	
	2/26	162/108	0.38	15.0	33	5.7	80	Discharged
			Delivered Feb. 14, 1932					
37	2/17	138/78	0.32	14.5	26	4.4	102	Aged 33 Para i
	2/2?	132/108	0.37	12 8	24	6.0	86	Discharged
38	2/26	175/120	0.36	11.0	26	5.9	61	Aged 24 Para i
	3/1	149/98	0.33	13.1	25	5.7	57	
	3/4	136/95	0.31	11.2	27	6.4	64	
	3/8	128/80	0.29	8.7	21	3.9	75	
	3/16	124/80	0.32	11.9	21	5.4	78	
	3/19	126/82	0.28					
			Delivered March 20, 1932					
	3/25		0.38	9.3	22		103	Discharged

Cases 5, 7, and 32). This hyperguanidinemia in cases with convulsions is especially interesting in view of the high values observed in other epileptiform states.[21, 22]

No direct relationship was observed between the blood pressures and the degree of hyperguanidinemia, although in general the cases with higher blood pressure showed higher guanidine values. Since the increase in blood guanidine always followed the rise in blood pressure, but did not precede it, it would appear that here as well as in essential hypertension (see Paper II of this series,[23] the increased guanidine content of the blood is not the cause of the hypertension.

In the one case of septicemia that was studied, there was no increase in the blood guanidine after delivery. This is in striking contrast to the condition of eclampsia, and even to that of the normal individuals after delivery.

Blood Guanidine in Cirrhosis of the Liver.—The results of blood guanidine determinations on three cases of portal cirrhosis of the liver are given in Table VI. The first two cases (diagnosed as alcoholic cirrhosis) show an increase in the blood guanidine concentration, but it should be noted that they also show a definite tendency to nitrogen retention, the urea nitrogen being above normal in all three determinations. It is, therefore, believed that the increased values shown in these cases are at least partially related to the renal insufficiency present. However, the third case, a patient whose condition was diagnosed as

TABLE VI

BLOOD GUANIDINE IN CIRRHOSIS OF THE LIVER

(Figures in mg. per 100 c.c.)

CASE	AGE AND SEX	UREA NITROGEN	SUGAR	BLOOD GUANIDINE	DIAGNOSIS
1	43M	21	93	0.38	Alcoholic cirrhosis
		17	98	0.35	Alcoholic cirrhosis
2	50M	21	96	0.40	Alcoholic cirrhosis
3	35M	7	92	0.40	Advanced Laennec's cirrhosis

advanced Laennec's cirrhosis of the liver, shows a guanidine value as high as or higher than the others, with the urea nitrogen definitely subnormal. While little can be concluded from the results of one determination, the hyperguanidinemia in this case lends some support to the findings of those investigators who have observed an increase in the blood guanidine concentration in marked kidney destruction.

SUMMARY

1. During normal pregnancy up to the time of delivery, the concentration of guanidine-like substances in the blood is essentially normal.

2. After delivery, the blood guanidine is increased to about double the antepartum value. The maximum concentration is reached within three to seven days after parturition, the value falling to practically normal by the end of the third week. This increase cannot be explained on the basis of blood concentration.

3. The increase in blood guanidine after delivery is mostly in the cells. The ratio of cell to plasma guanidine shows an average value of 5.5 instead of the normal figure of 2.4.

4. Blood guanidine concentration in mild cases of toxemias of pregnancy is practically the same as in the normal maternity cases. In severe toxemias, the blood guanidine may show an additional rise, the high value remaining as long as the toxic state exists. Often this increase is accompanied by a tendency for nitrogen retention. Distinctly high figures were found in all cases with tetanic symptoms.

5. Blood guanidine was found to be slightly increased in one case of advanced portal (Laennec's) cirrhosis of the liver.

REFERENCES

1. Minot, A. S., and Cutler, J. T.: Guanidine Retention and Calcium Reserve as Antagonistic Factors in Carbon Tetra-Chloride and Chloroform Poisoning, J. Clin. Investigation **6**: 367, 1928.
2. Major, R. H., and Weber, C. J.: The Probable Presence of Increased Amounts of Guanidine in Blood of Patients with Arterial Hypertension, Bull. Johns Hopkins Hosp. **40**: 85, 1927.
3. Minot, A. S., and Cutler, J. T.: Increase in Guanidine-Like Substance in Acute Liver Injury, Proc. Soc. Exper. Biol. & Med. **26**: 607, 1929.
4. Major, R. H., and Weber, C. J.: Probable Increase of Guanidine in the Blood of Certain Persons with Hypertension, Arch. Int. Med. **40**: 891, 1927.
5. Ellsworth, R.: Guanidine Base Concentration in the Blood of Normal Individuals and in Patients with Severe Liver Injury, Bull. Johns Hopkins Hosp. **46**: 296, 1930.
6. Ellsworth, R. M.: Response of Blood Guanidine Base Concentration of Normal Individuals and of Patients with Liver Injury, to the Injection of Methyl Guanidine Sulfate, Bull. Johns Hopkins Hosp. **47**: 106, 1930.
7. Laffont, A.: Malméjac, R., and Sirjean: Guanidinemia and Gestation, Ann. de méd. Physiol. **6**: 395, 1930.
8. Malméjac, J.: Guanidinemia et d'intoxication gravidique, Rev. franç. de gynéc. et d'obst. **25**: 435, 1930.
9. Malméjac, R.: Las rapports de la guanidinemia avec la puerperalité, Gynec. et obst. **24**: 689, 1931.
10. Cutler, J. T.: Accumulation of Guanidine in the Blood Following Acute Liver Injury by Carbon Tetrachloride, Chloroform, Arsenic or Phosphorus Poisoning, J. Pharmacol. & Exper. Therap. **41**: 337, 1931.
11. Arkawa, M.: Über die Beziehungen der Leberfunktion zu der im Blut enthalten Guanidin- und Blutzuckermenge, Nagoya J. M. Sc. **6**: 96, 1932.

12. Minot, A. S., and Dodd, K.: Guanidine Intoxication: a Complicating Factor in Certain Clinical Conditions in Children, Am. J. Dis. Child. **46**: 522, 1933.
13. Zappacosta, M., and Colorusso, A.: La metilguanidina come di esplorazione della funzionalità epatica, Diag. e tec. di lab. **6**: 801, 1935.
14. Remond, A., and Colombies, R.: Investigaciones sobre el metabolismo de la guanidina, Ars Med. Barcelona **6**: 71, 1930.
15. Hepler, O. R., and Simmonds, J. P.: The Effect of Mechanical Obstruction of the Hepatic Veins Upon the Guanidine Content of the Blood, Proc. Soc. Exper. Biol. Med. **28**: 583, 1931.
16. de Wesselow, O. L. V. S., and Griffith, W. J.: Blood Guanidine in Hypertension, Brit. J. Exper. Path. **13**: 428, 1932.
17. Turries, Jean: Sur la guanidine du samg dans les affections hépatiques et l'insuffisance renal, Compt. rend. Soc. de biol. **112**: 658, 1933.
18. Stander, H. J.: Guanidine Base Concentration in Eclampsia, Am. J. Obst. & Gynec. **23**: 373, 1932.
19. Krieger, V. I.: Observations on Guanidine, Glucose and Calcium Content of Blood in Eclampsia, M. J. Australia **2**: 746, 1934.
20. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in Blood. I. Colorimetric Estimation and Normal Values, J. Lab. & Clin. Med. **22**: 1147, 1937.
21. Ellis, M. M., Neal, M. P., and Frazier, T. R.: Guanidine Content of Blood From Epileptics, Proc. Soc. Exper. Biol. & Med. **28**: 553, 1931.
22. Pekelis, E., and Parenti, P.: Recerche sulla guanidinemia. Ulteriore contributo alla conoscenza del comportamento clinico della iperguanidinemia, Riv. di clin. med. **35**: 849, 1934.
23. Andes, J. E., Linegar, C. R., and Myers, V. C.: Guanidine-Like Substances in the Blood. II. Blood Guanidine in Nitrogen Retention and Hypertension, J. Lab. & Clin. Med. **22**: 1209, 1937.

CALCIUM TOLERANCE CURVES IN PAGET'S DISEASE OF THE BONE*

Isabel M. London, M.D., and Alice R. Bernheim, M.D.,
New York, N. Y.

IN A SERIES of 146 cases, including 17 cases of untreated Paget's disease of the bone and 22 normal subjects, 201 calcium tolerance tests were made according to a technic developed during the past four years at the New York Hospital.

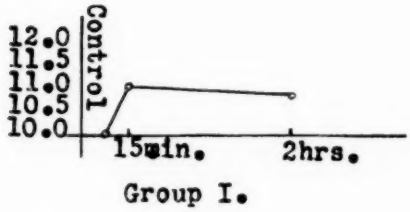
Technic.—Subjects were instructed to come to the hospital fasting, and were given chocolate, one-half to one ounce, to eat. This was done in order to avoid unpleasant reactions which are likely to follow the intravenous administration of calcium to a fasting subject. Five to ten minutes thereafter an initial blood specimen was withdrawn, and a uniform test dose of 10 c.c. of 20 per cent calcium gluconate solution† (0.186 grams of calcium) was injected slowly, during four to five minutes. First and second specimens were withdrawn 15 minutes and two hours after the injection for analysis.

Methods.—Determinations were made for calcium, phosphorus, and phosphatase in the serum. Calcium was determined by the Kramer-Tisdall method, phosphorus by the Bodansky modification of the Kuttner-Lichtenstein method, and phosphatase by the Bodansky method.

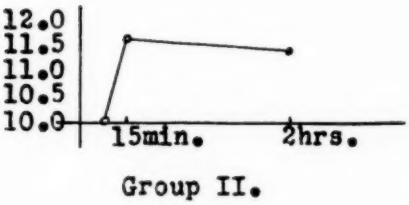
*From the New York Hospital, Department of Surgery, and Cornell University Medical College.

†Calglucon, Sandoz.

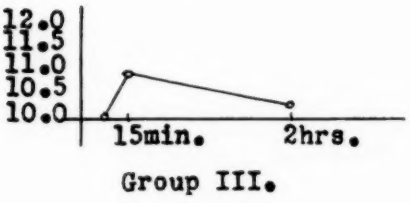
Received for publication, November 2, 1936.



Group I. Rise in calcium of 0.5 mg. to 1.4 mg. per cent inclusive.
Fall in calcium of less than 0.5 mg. per cent from peak at end of two hours.

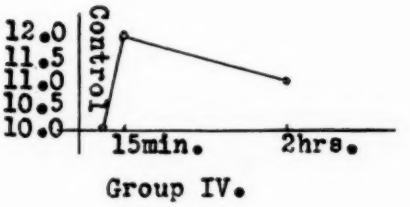


Group II. Rise in calcium of 1.5 mg. per cent or over.
Fall in calcium of less than 0.5 mg. per cent from peak at end of two hours.

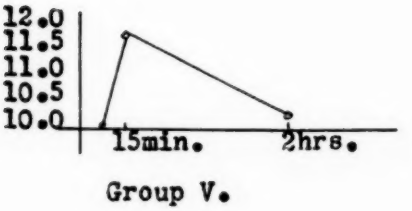


Group III. Rise in calcium of 0.5 mg. to 1.4 mg. per cent inclusive.
Fall in calcium of 0.5 mg. to 1.4 mg. per cent from peak at end of two hours.

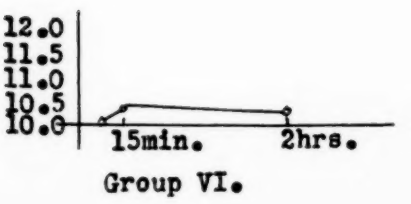
Chart 1.



Group IV. Rise in calcium of 1.5 mg. per cent or over.
Fall in calcium of 0.5 mg. to 1.4 mg. per cent inclusive from peak at end of two hours.



Group V. Rise in calcium of 1.5 mg. per cent or more.
Fall in calcium of 1.5 mg. per cent or more from peak at end of two hours.



Group VI. Rise in calcium of less than 0.5 mg. per cent.
Fall in calcium of less than 0.5 mg. per cent from peak at end of two hours.

Chart 2.

Results.—The calcium curves obtained were grouped into six categories, as shown in Figs. 1 and 2.

Curves in Groups I and III are characterized, therefore, by a moderate rise from the initial level, and in II, IV, and V by a marked rise. Curves in Groups I and II are characterized by an insignificant fall from the peak within two hours, in III and IV by a moderate fall (0.5 mg. per cent to 1.4 mg. per cent), and in V by a rapid fall. Curves in Group VI are characterized by an insignificant rise and fall approaching a flat type.

The curves obtained fell into these six groups as follows:

I	II	III	IV	V	VI
38	16	43	55	14	7

The remaining 28 curves of the total of 201 tests were duplicate curves, occurring in the same patient on two or more occasions, and were therefore not included. Of those included, 27 curves represented a change in type of curve in the same patient on repetition, after treatment.

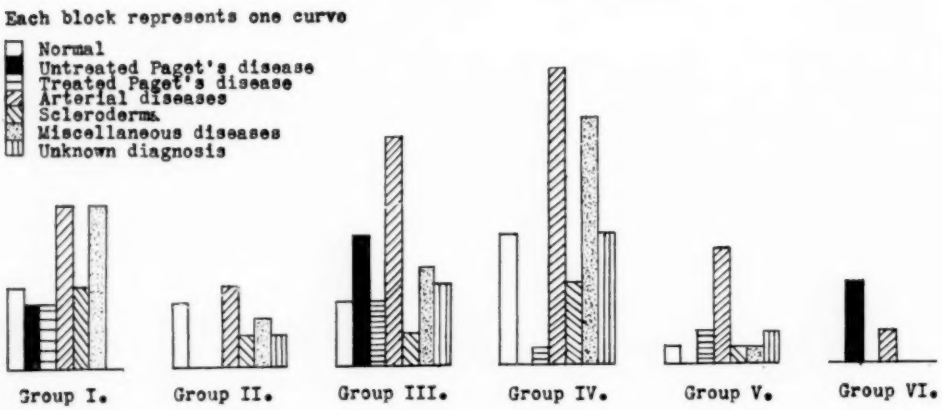


Chart 3

As may be seen in Chart 1, a fairly uniformly proportionate distribution of curves occurred in Groups I and III for normal subjects, Paget's disease and miscellaneous conditions. On the other hand, a marked discrepancy occurred in the remaining groups between the curves of Paget's disease and all the rest. Thus not a single case of untreated Paget's disease is to be found in Groups II, IV, and V, and only three treated cases appeared in these groups, whereas in Group VI, 5 of the 7 cases were Paget's disease.

It appears then that the calcium curve of Paget's disease is notable for an absence of marked deviation from the pre-injection level. The disparity with the normal is brought out best in the following:

Group	I	II	III	IV	V	VI
Paget's	4	0	8	0	0	5
Normals	5	4	4	8	1	0

Thus Groups II, IV, and V (rise of calcium of 1.5 mg. per cent or more), include 13 of a total of 22 normals and no untreated Paget's cases. Group VI

(rise and fall of less than 0.5 mg. per cent) contained 5 out of the entire group of 17 untreated Paget's curves, and no normals.

The phosphorus curves showed a significant rise or fall in a few instances only, and could not be correlated with the calcium curve or the condition. The phosphatase curves showed no significant variations, remaining low in the normals (2.1 to 4.4) and moderately to markedly elevated in the cases of Paget's disease (6.8 to 100 units). No correlation was observed between the height of the phosphatase and the type of calcium curve.

Comment.—The curve obtained in Paget's disease, in view of its tendency to resist marked upward deviation, would appear to indicate an increased affinity for calcium on the part of the bones and other tissues, or a decreased affinity for calcium on the part of the blood. The former interpretation is in line with the metabolic studies reported by several investigators,[1-5] showing a retention of calcium by the body in Paget's disease of the bone.

In this connection it may be interesting to note briefly the results of our metabolic study of a case of Paget's disease observed over a period of two months in 1930. This patient also showed a greater retention of calcium than the normal. When on a low calcium intake (approximately 0.133 gm. daily), he lost 0.208 gm. daily, a loss of 158 per cent. According to Bauer and Aub,[6] the normal on an equivalent intake (0.110 gm.), loses 0.263 gm. daily, a loss of 221 per cent.

On increasing levels of calcium intake the negative balance undergoes inversion toward the positive, as in the normal, but to a greater extent. Whereas, in the normal, Sherman[7] found that an intake of 1.9 gm. of calcium daily resulted in a retention of 0.400 gm. (21 per cent), in the Paget's case studied an intake of 2.03 gm. of calcium daily resulted in a retention of 0.756 gm. (37.2 per cent). These figures parallel those reported by Rabinowitch,[5] who found a retention of 40.2 per cent with a daily intake of 2.45 gm. of calcium in Paget's disease.

The subject of this metabolic study, when tested for calcium tolerance about four years later, showed the curve characteristic of Group VI, i.e., an almost flat curve.

In the literature only one report of calcium tolerance curves in Paget's disease has been found.[8] Three curves were reported (Lasch); two showed a rise of 1.4 mg. per cent and 1.3 mg. per cent, thereby falling into Group III, where 8 of our 17 untreated cases appeared. The third case showed a rise of 1.8 mg. per cent from an initial calcium of 12.2 mg. and fall of 2.0 mg. per cent, thereby falling into Group V, where none of our Paget's cases appeared.

SUMMARY

The serum calcium response to injection of calcium gluconate intravenously was studied in 17 cases of untreated Paget's disease. The resulting curves showed a tendency to smaller deviations than those found in normal controls and in a series of miscellaneous conditions. In five cases of Paget's disease an almost flat curve was obtained. This was not obtained in any of the normal subjects.

REFERENCES

1. Da Costa, Funk, Bergeim, and Hawk: Osteitis Deformans, Proc. Am. Soc. Biol. Chem., 1914, xvii, p. 30.
2. Cuthbertson, D.: Balance of Calcium, Magnesium, Phosphorus, Sulphur in a Case of Osteitis Deformans, Glasgow M. J. **108**: 218, 1927.
3. Van Hazel, W., and Andrews, E.: Osteitis Deformans, Surg. Gynec. Obst. **45**: 54, 1927.
4. Labbé, M., Nepveux, F., Boulin, R., and Escalier, A.: Phosphorus and Calcium Metabolism in Osteitis Deformans, Comp. rend. Soc. de biol. **109**: 1110, 1932.
5. Rabinowitch, I. M.: Metabolic Studies in a Case of Osteitis Deformans, J. Nutrition **5**: 325, 1932.
6. Bauer, M., and Aub, J. C.: Studies of Inorganic Salt Metabolism. I. The Ward Routine and Methods, J. Am. Dietetic Assn. **3**: 1927.
7. Sherman, H. C., Mettler, A. J., and Sinclair, J. E.: Calcium, Magnesium, and Phosphorus in Food and Nutrition, U. S. Dept. Agric. Office Exper. Sta., Bull. 227, p. 31, 1910.
8. Lasch, F.: Biochemical Studies in Paget's Osteitis Deformans, Wien. Arch. f. innere Med. **21**: 1931.

THE ABSORPTION SPECTRA OF THE DIRECT AND INDIRECT REACTING TYPES OF SERUM BILIRUBIN*

GEORGE E. DAVIS,† PH.D., AND
CHARLES SHEARD, PH.D., ROCHESTER, MINN.

MANY attempts have been made to explain why serum bilirubin shows two principal types (direct and indirect) of reaction when various samples are subjected to the van den Bergh test with sulphanilic acid and sodium nitrite. In obstructive jaundice the reaction is usually prompt or "direct"; in hemolytic jaundice, typically delayed or "indirect." It is not possible in this paper to review the numerous researches bearing upon such differences in reaction or the various theories proposed to account for them. Hunter[6] has given a brief review of the more important investigations and theories to 1930, together with a summary of the principal chemical, physical and physiologic evidence concerning the existence of two types of bilirubin. We shall confine ourselves to a brief consideration of the investigations involving spectrophotometric methods.

Magath and Sheard[9] made spectrophotometric analyses of normal human blood serums and of serums of patients who had malaria, pernicious anemia, hemolytic jaundice and obstructive jaundice. Values of transmission in the spectral range from 700 to 430 mμ were obtained by means of a visual spectrophotometer, the Keuffel and Esser "color analyzer." No differences were found in the types of spectrophotometric curves obtained from diluted serums exhibiting the direct and indirect types of reaction. It was concluded that if the bilirubin contained in blood serums differs, it cannot be shown spectrophotometrically. This conclusion was applicable only to the visible spectrum in the range in which the observations were made (700-430 mμ) and to serums diluted with a mixture of alcohol and acetone.

*From the Division of Physics and Biophysical Research, The Mayo Foundation.
Received for publication, February 14, 1937.

†Formerly a fellow in the Mayo Foundation. Now associate in the Department of Physics, Duquesne University, Pittsburgh, Pa.

Heilmeyer and Krebs[3] prepared diazotized alcoholic extracts of serum containing the direct and indirect types of bilirubin, using the method of Thannhauser and Andersen. They compared the absorption spectra of these extracts in the visual range from 650 to 470 mμ, using a König-Martens spectrophotometer. They considered the spectrophotometric curves to be almost identical in shape, although certain definite differences were evident, particularly in the spectral region between 575 and 470 mμ where the curves diverge greatly. Furthermore, the types of curve obtained also differ from the curve for azopigment in chloroform and alcohol, formed by diazotizing purified bilirubin. The authors concluded from the similarity of the spectra that the two forms of bilirubin discussed by van den Bergh give identical azopigments and that differences in the bilirubin molecule are not responsible for variations in the diazo reaction.

Müller and Engel[10] stated that their experiments with serums in cases of icterus confirm the conclusion of Magath and Sheard that the absorption spectra of direct and indirect reacting bilirubins are the same. They stated that the spectra agree completely, and also agree with the spectrum of normal serum to which bilirubin has been added artificially. The spectrophotometric evidence upon which this conclusion is based is not presented. They also prepared diazotized solutions (method of Thannhauser and Andersen) of bilirubin in chloroform, of normal serum with bilirubin added artificially, and of direct- and indirect-reacting serums in cases of icterus. Absorption spectra of these solutions were obtained in the spectral region between 650 and 470 mμ, using a König-Martens spectrophotometer. It is shown that the curves of spectral absorption can be made to coincide almost exactly from 650 mμ to beyond the wave length of maximal absorption, which is approximately 575 mμ. The four curves begin to diverge at about 550 mμ and spread farther and farther apart as wave length 470 mμ is approached. According to Müller and Engel, these differences are due to the presence of different amounts of uncoupled bilirubin. This explanation is based upon the statement of Fischer and Barrensheen (1921) that the coupling in the diazo reaction is never complete. The authors concluded that the bilirubins in direct- and indirect-coupled serums exhibit no spectral differences or changes in crystalline form. It is to be noted, however, that the spectrophotometric evidence as presented does not warrant this conclusion being applied to the bilirubins as they occur naturally in blood serum, since it is not proved that any chemical or physical differences which may exist are not destroyed during preparation of the diazotized solutions. The conclusion must be taken to apply only to the bilirubins after they have been changed to the azopigments.

Heilmeyer and Toop[4] obtained a considerable number of visual absorption spectra of pigments occurring in blood serum, including bilirubin in the following natural and artificially prepared solutions: natural serum bilirubin produced by hemolysis in vivo, induced by injection of distilled water; human serum with bilirubin injected in vivo; purified bilirubin in various solvents, including blood serum; human bile; serums from patients with icterus, icterus catarrh, and hemolytic icterus. The spectra are represented by straight-line curves only, with

the lower limit at 420 mμ or above. The authors do not draw any conclusions concerning the possibility of distinguishing between the two van den Bergh types of bilirubin spectrophotometrically. It is unlikely that any general differentiation could be made from the curves they have presented.

In these researches, the absorption spectra were examined only in the visible spectrum, the lower limits of wave length being: Magath and Sheard, 430 mμ; Heilmeyer and Krebs, and Müller and Engel, 470 mμ; Heilmeyer and Toop, 420 mμ or above. These observations cover approximately only half of the broad characteristic absorption band of bilirubin, which extends well into the ultraviolet. In the present investigations we have measured the absorption of the two types of serum bilirubin over the entire band, examining the spectra of various samples more critically and comparing the spectra of the two types of bilirubin with greater quantitative accuracy than has been evidenced in previous investigations.

EXPERIMENTAL PROCEDURE

The absorption spectra of a number of samples of human serum in cases of icterus were obtained. Some of the serums gave the direct, some the indirect, type of van den Bergh reaction. It should be borne in mind that serums containing direct-reacting serum bilirubin also may and usually do contain a varying amount of indirect-reacting bilirubin. Since it is impossible to separate these two types, curves plotted from determinations made on the direct type represent results obtained on mixtures of this type and the indirect type. Hence in referring to spectrophotometric data obtained from direct-reacting serums, it is to be understood that all the bilirubin in these serums was not of the direct-reacting type.

The spectra were represented by plotting transmissions against wave lengths. The experimental procedure was as follows: About 10 c.c. of blood was obtained from a patient known to have pronounced jaundice, care being taken to avoid hemolysis so far as possible. The blood was at once put into a refrigerator to clot. After about one hour the clot was carefully loosened and the blood was centrifuged. The serum was returned to the refrigerator or put into a Dewar flask containing ice at 0° C., where it was kept until examined spectrophotometrically. An estimation of the concentration of bilirubin was made on a portion of the serum by the van den Bergh method.

The spectrophotometric examinations of the serums were made by means of a large quartz spectrograph (photographic plates 2.6 by 10 inches) and a rotating sector photometer, employing an under-water spark between tungsten terminals as the source of radiation. The instruments were assembled and used in the usual manner, the method being essentially as first described by Howe.[5] The electrical circuits, quartz cells and container for the under-water spark have been described elsewhere by the authors.[1]

Spectroscopic examination showed that most blood serums, however carefully prepared, contained oxyhemoglobin sufficient in amount to affect the absorption spectrum even though the serum appeared to the unaided eye to be almost or quite free from red color. Bilirubin could not be removed from the serum with the assurance that the spectral transmission of the other components

would remain unchanged. Because of this fact we could not use bilirubin-free serum as the comparison fluid. Therefore distilled water was used instead and correction was made for the absorption in the oxyhemoglobin when traces of that pigment were present. The corrections involved the following steps: (1) estimation of the concentration of oxyhemoglobin in the serum; (2) calculation of the curve of spectral transmission of the oxyhemoglobin alone; (3) correction of the observed transmission curve of the serum by dividing the transmission, at each of a sufficient number of wave lengths, by the corresponding transmission of the oxyhemoglobin, as shown by the calculated curve. The details of this method of correcting for traces of oxyhemoglobin have been described previously.[2]

The absorption spectra of six serums giving the direct van den Bergh reaction were determined. We were able to obtain only a few good serums showing the indirect reaction and having a bilirubin concentration high enough to give an absorption spectrum relatively little affected by absorption in components of the serum other than traces of oxyhemoglobin. Reliable transmission curves were obtained for three serums which gave the indirect reaction. Six of the nine serums were examined without dilution or the addition of any substance. Three serums were diluted to one part in three by adding a 0.1 per cent solution of sodium carbonate. In every case but one the serums were examined in a quartz cell 0.77 mm. thick; in the one case the thickness was 5.84 mm. The absorption spectra of the serums were represented by plotting transmissions against wave lengths.

ABSORPTION SPECTRA OF SERUM BILIRUBIN

Four of the spectra are shown in Fig. 1 and illustrate the typical characteristics and the variations obtained. The concentrations of bilirubin were determined by the van den Bergh method: the data are given in the legend accompanying the curves.

Before comparing the curves, it is to be noted that the absorption band at 280 mμ, Curve 2, is not characteristic of bilirubin but is due to serum proteins, as shown by Lewis.[8] Although Curves 1, 3, and 4 are not extended far enough to show this band, nevertheless the shapes of the curves below 330 or 340 mμ are affected by it. For this reason the curves will be compared only in the spectral region above about 340 mμ.

Marked variations in the spectrophotometric transmissions, even for bilirubins of the same type, are quite apparent. Significant differences in absorption are shown by the major contours of the curves. For example, in Curves 2, 3 and 4, representing nearly equal concentrations of bilirubin (Curve 2, direct; Curves 3 and 4, indirect), the transmissions are very nearly equal at wave length 495 mμ but differ widely throughout most of the spectral range of the measurements. Curves 2 and 4 cross at 495 mμ and practically coincide from 335 to 360 mμ; but the transmission shown in Curve 4 is about 29 per cent greater at 410 mμ and 16 per cent less at 530 mμ. That these differences are not necessarily chiefly due to the difference in type of bilirubin, however, is shown by the fact that similar large differences in transmission, varying in sense (positive or negative) one or more times along the spectral ranges represented,

are seen in comparing Curves 2 and 3 (direct and indirect) and Curves 3 and 4 (both indirect). The absorption spectra of bilirubins of the same van den Bergh type often differ as markedly as they differ from the spectra of bilirubins unlike them in type. The differences are commonly many times larger than the greatest possible experimental error of the measurements.

Distinct minor variations in the spectra of both types of bilirubin also were observed, and they are illustrated in the curves of Fig. 1. These variations are doubtless genuine, since the experimental errors are generally too small to be largely responsible for them. The observed values of transmission, uncorrected for absorption due to oxyhemoglobin, are correct to within approximately 1 per cent transmission. Occasionally the errors in the corrected curves may be greater in cases in which the corrections are large, but they should seldom

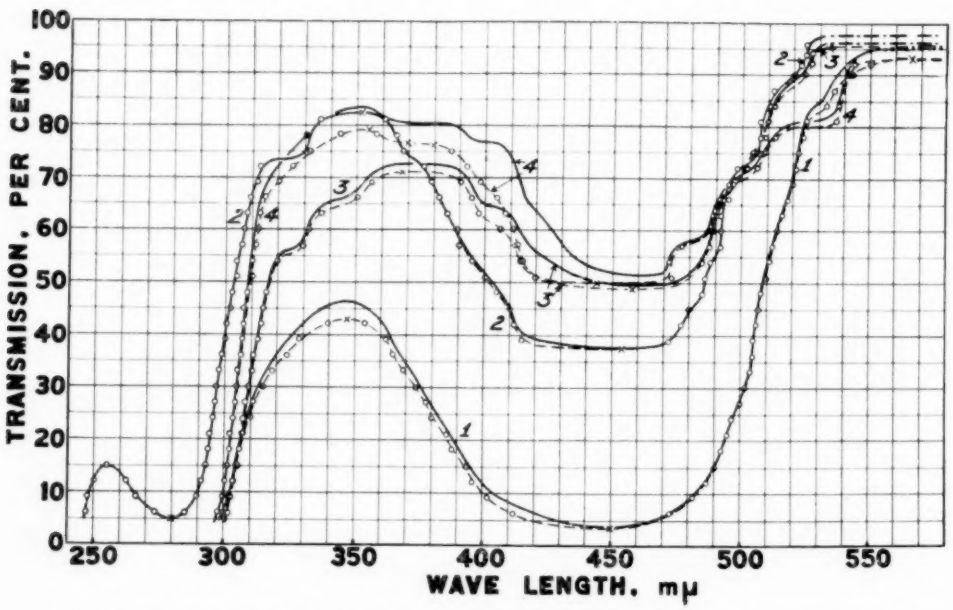


Fig. 1.—Transmission curves of blood serum from patients. These curves are typical for serum bilirubin in the spectral region above about 340 mμ. Dotted curves, observed transmissions; full line curves, after correction for absorption by traces of oxyhemoglobin. Types of reaction and concentrations of bilirubin (mg. per 100 c.c.) as determined by the van den Bergh method: Curve 1, direct, 22.3; Curve 2, direct, 5.0 (original serum 15.0, here reduced by dilution); Curve 3, indirect, 4.5; Curve 4, indirect, 4.3. Thickness of spectroscopic cell, 0.77 mm. in each case.

exceed 2 per cent. We should not expect the errors to vary abruptly in short spectral ranges in such a manner as to cause the type of wavy or steplike sections shown in certain portions of the curves. Similar variations in or near the absorption band at 280 mμ, were observed by Lewis[7] in the absorption spectra of blood serums, and later[8] in the spectra of euglobulin, pseudoglobulin and albumin. The curves were described by Lewis as having steplike irregularities which varied with the pathologic conditions of the subjects (human) from whom the serums were obtained.

These minor variations in the spectra undoubtedly are due mainly to differences in chemical composition, either in the bile pigment or in the other

components of the serum, or in both. Since water was used as the comparison substance, the spectral curves show the absorption not only of bilirubin but of all the other serum components except oxyhemoglobin. If it had been possible in each case to remove the bilirubin from some of the serum without affecting the other components, and to use this bilirubin-free serum as the comparison substance, the spectral curves would show only the absorption by the bilirubin.

It is evident that any differences which may occur in the absorption spectra, as between the direct and indirect reaction types of bilirubin, are not revealed on gross examination of the curves but are masked by the individual variations which occur in the spectra of both types. Therefore, in order more adequately to analyze and compare the spectra, we have recorded and averaged in Table I the wave lengths of maximal absorption in the typical bilirubin band, the wave lengths of minimal absorption adjacent to this band on the short-wave side, and the values of six ratios of optical densities. The optical density D is the negative logarithm of the transmission, to base 10. The ratio of the optical densities corresponding to any two wave lengths in the absorption spectrum of a substance is a constant, regardless of the concentration or the thickness of solution examined. Hence in comparing the spectra of two substances, any differences in corresponding ratios of optical density may be considered evidence that the substances are not identical. All of the ratios here given involve densities in the characteristic absorption band of bilirubin.

The values of the wave length of maximal absorption do not vary greatly, considering that the absorption band is broad and nearly "flat" in that region, making it more difficult to determine the position of the maximal accurately. The average wave length of maximal absorption for the indirect bilirubins, 459.3 mμ, is about 10 mμ greater than the value for the direct bilirubins, 449.5 mμ.

The wave lengths of minimal absorption adjacent to the bilirubin band vary somewhat more widely from sample to sample. The difference of about 8

TABLE I

CONSTANTS OF THE ABSORPTION SPECTRA OF SAMPLES OF DIRECT AND INDIRECT SERUM BILIRUBIN

TYPE OF VAN DEN BERGH REACTION	CONCENTRATION OF BILIRUBIN, MG. PER 100 C.C.	WAVE LENGTH OF MAXIMUM ABSORPTION, Mμ.	WAVE LENGTH OF MINIMUM ABSORPTION Mμ.	RATIOS OF OPTICAL DENSITIES (D)*					
				$\frac{D_{min}}{D_{max}}$	$\frac{D_{400}}{D_{max}}$	$\frac{D_{490}}{D_{max}}$	$\frac{D_{510}}{D_{max}}$	$\frac{D_{490}}{D_{400}}$	$\frac{D_{510}}{D_{400}}$
Direct	23.7	448	347	0.27	0.65	0.57	0.23	0.88	0.34
Direct	22.3	450	347	0.23	0.64	0.56	0.19	0.88	0.29
Direct	5.0	455	352	0.26	0.66	0.66	0.24	1.01	0.37
Direct	5.0	454	352	0.19	0.68	0.60	0.17	0.89	0.25
Direct	3.3	450	362	0.46	0.59	0.61	0.36	1.04	0.61
Direct	1.7	440	361	0.54	0.64	0.80	0.45	1.25	0.70
Indirect	4.3	460	354	0.27	0.40	0.65	0.40	1.62	0.99
Indirect	1.0	458	(?)†	0.35	0.35	0.82	0.50	2.34	1.43
Indirect	4.5	460	372	0.46	0.62	0.66	0.29	1.07	0.47
Average, directs		449.5	355.2	0.32	0.64	0.63	0.27	0.99	0.43
Average, indirects		459.3	363.0	0.36	0.46	0.71	0.40	1.68	0.96
Average, all		452.8	357.1	0.34	0.58	0.66	0.31	1.22	0.61

*D_{max} and D_{min} are the optical densities at the wave lengths of maximal and minimal absorption, respectively. The numerical subscripts 400, 490, and 510 indicate spectral positions at these wave lengths (mμ).

†Value not definitely indicated by the curve.

mμ between the average values for the two types of pigment is no greater than we should expect from the individual variations. It is to be noted, however, that the value for the indirect bilirubins is the higher, as is the case for the values at maximal absorption.

The ratios of optical densities shown in the table must be examined carefully in deciding whether there are any real differences between the average values for the direct and indirect-reacting types of bilirubin. Considering the magnitude of the variation in each ratio from sample to sample of serum, the differences in the average values seem significant except for D_{min}/D_{max} and D_{490}/D_{max}. The data therefore indicate that, on the average, D_{400}/D_{max} is greater, while D_{510}/D_{max}, D_{490}/D_{400} and D_{510}/D_{400} are less, for the direct reaction type of bilirubin than for the indirect type. Considering that the reciprocal of the first quantity is less for the direct types, these results mean that the absorption at 400 mμ, as compared to the absorptions at the maximum and at 490 and 510 mμ, is greater for the direct reaction type than for the indirect. The absorption at the maximum, as compared to that at 510 (and possibly at 490) mμ, also is greater for the direct types. In speaking of transmissions, these statements should be reversed. These data are consistent, indicating that the typical bilirubin absorption band lies farther toward the shorter wave lengths when the reaction is direct than when it is indirect. This is shown also by the fact that the average wave length of maximal absorption is about 10 mμ less for the direct reaction pigment. The average wave length of minimal absorption adjacent to the band appears to be similarly shifted, as has been noted. While these results are clearly indicated, they are not conclusive, since the number of samples of serum examined is small and the variations in any one absorption ratio are relatively large. A larger number of samples should be examined. The average values of the absorption ratios shown in Table I are approximate characteristic constants of the spectra of the two forms of serum bilirubin.

While it cannot be stated positively that the presence of indirect-reacting bilirubin in serum containing direct-reacting pigment changes to any marked degree the type of the curve, it seems likely that the difference in the absorption spectra between direct and indirect-reacting bilirubin is actually greater than is here indicated, and that if a serum containing only direct-reacting bilirubin were obtained, the maximal absorption would be obtained at a wave length shorter than we have found. It will be appreciated that the problem is complicated by this situation and that, in reality, we were comparing the spectra of indirect-reacting bilirubin (in serum) with a serum containing a mixture of direct- and indirect-reacting bilirubin in which the proportion of the two was unknown.

SUMMARY

The absorption spectra of a number of samples of serum bilirubin (showing the direct and indirect types of van den Bergh reaction, respectively) were obtained by means of the quartz spectrograph and a sector photometer. Typical spectral transmission curves are shown and discussed. The absorption spectra of different specimens vary markedly, even for bilirubins of the same type.

These variations tend to mask any differences in spectra which may be due to a difference in type of pigment. By comparing various absorption ratios and the wave lengths of maximal and minimal absorption, it is shown that the spectrum of serum containing direct-reacting bilirubin differs from that of the indirect type in that its characteristic absorption band is shifted about 10 mμ farther toward the shorter wave lengths. A similar difference of about 8 mμ is indicated for the wave lengths of minimal absorption adjacent to the band. In serum, the average values of the wave lengths of maximal absorption were found to be approximately 449 mμ for the direct-reacting serum bilirubins and 459 mμ for the indirect type; the minimal absorption in the ultraviolet region of the spectrum of serum bilirubin occurred at about 355 mμ for the direct type and at 363 mμ for the indirect type. These data suggest that the two types of serum bilirubin may be distinguished spectrophotometrically if methods adequate for such determinations in the violet and ultraviolet portions of the spectrum are employed. However, the problem is complicated by the presence of indirect-reacting bilirubin in serum containing direct-reacting pigment.

REFERENCES

1. Davis, G. E., and Sheard, Charles: Ultraviolet Spectrophotometry: Comments on Apparatus and Experimental Methods, J. Optic Soc. America **21**: 47, 1931.
2. Davis, G. E., and Sheard, Charles: The Spectrophotometric Investigation and Determination of Bilirubin, J. LAB. & CLIN. MED. **19**: 593, 1934.
3. Heilmeyer, Ludwig, and Krebs, Willy: Spektrophotometrische Untersuchungen des Ehrlich-Pröscherschen Bilirubin-Azofarbstoffes und ihre praktische Anwendung, besonders zur quantitativen Bestimmung des Bilirubins im Blutserum, Biochem. Ztschr. **223**: 352, 1930.
4. Heilmeyer, L., and Toop, H.: Klinische Farbmessungen. XII. Mitteilung. Die Spektrophotometrische Farbstoffanalyse des Blutserums mit besonderer Berücksichtigung des Bilirubin- und Carotinspektrums, Ztschr. f. d. ges. exper. Med. **80**: 603, 1932.
5. Howe, H. E.: On a Modification of the Hilger Sector Photometer for Measuring Ultraviolet Absorption and Its Application in the Case of Certain Derivatives of Fluoran, Physical Rev. **8**: 674, 1916.
6. Hunter, George: On the Two Types of Bilirubin Diazo-Reaction in Serum, With a Hypothesis on the Nature of the Bilirubin in the Serum From Haemolytic Jaundice, Brit. J. Exper. Path. **11**: 415, 1930.
7. Lewis, S. J.: The Ultra-Violet Absorption Spectra of Blood Sera, Proc. Roy. Soc. London **89**: 327, 1916.
8. Lewis, S. J.: The Ultra-Violet Absorption Spectra and the Optical Rotation of the Proteins of Blood Sera, Proc. Roy. Soc. London **93**: 178, 1922.
9. Magath, T. B., and Sheard, Charles: Spectrophotometric Analysis of Blood Serum in Normal and Pathologic Conditions. Study I, Arch. Int. Med. **39**: 214, 1927.
10. Müller, P., and Engel, L.: Über das Absorptionsspektrum des Bilirubins in verschiedenen Lösungsmitteln. III. Mitteilung, Hoppe-Seyler's Ztschr. **202**: 56, 1931.

ANAPHYLAXIS IN DECEREBRATED MONKEYS*

LEO M. DAVIDOFF, M.D., NICHOLAS KOPELOFF, PH.D., AND
LENORE M. KOPELOFF, A.M., NEW YORK, N. Y.

THERE is conflicting evidence as to whether or not anaphylactic shock can occur in the absence of the cerebrum. Pearce and Eisenbrey,[3] Schürer and Strasmann,[4] and Zunz and La Barre[5] reported anaphylactic shock following extirpation of the brain, while Lumière and Couturier[2] found that the cerebral centers were indispensable. Guinea pigs, rabbits, and dogs were used as experimental animals in the above investigations.

Since the brain of the monkey resembles that of man more closely, 4 Macacus rhesus monkeys previously sensitized to egg white were decerebrated and the same antigen subsequently injected. As controls 2 egg white sensitive animals not subjected to the operative procedure were injected with the test antigen as in our previous report.[1] Further, 2 normal (unsensitized) monkeys were decerebrated and later injected with the test antigen.

Using light ether anesthesia, the entire cerebrum was amputated just anterior to the midbrain. This procedure was carried out under direct vision by means of a sharp scalpel. Bleeding from the basilar and carotid arteries was stopped immediately with hemostats. The animals were covered with warmed cotton and in a few instances artificial respiration was used for a short time. The next step in the experiment was delayed in each case until spontaneous respiration was well established.

Seven to twenty-nine minutes after decerebration the monkeys were injected intravenously with 8 or 10 c.c. of the test antigen (fresh undiluted egg white). Prior to this, 4 received an intravenous injection of 10 c.c. of physiologic saline five to nineteen minutes after the removal of the cerebrum. In no case did the saline cause any observable reaction, the respirations continuing at about the same rate. This contrasted with the dramatic, immediate anaphylactic reaction which occurred when egg white was administered to the sensitive animals.

RESULTS

Removal of the cerebrum from 4 egg white sensitive monkeys failed to prevent anaphylaxis. Anaphylactic death occurred in from ten seconds to six minutes. The 2 sensitized controls also died of anaphylactic shock in two to four minutes as a result of the test injection.

In spite of the major trauma of complete decerebration it was possible to keep 2 unsensitized monkeys alive for about one and one-half hours. The rate of respirations remained approximately the same in these animals before and after the intravenous injection of 8 or 10 c.c. of egg white.

*From the Department of Bacteriology, New York State Psychiatric Institute and Hospital.

Received for publication, March 9, 1937.

The 6 sensitized animals (2 controls) all died within six minutes following the test dose. In the 4 decerebrated sensitized animals as well as in the 2 controls, typical anaphylactic shock occurred as follows: immediate marked increase in the rate of respirations after the injection of antigen; gasping; contraction and stiffening of the arms and legs; death.

A representative protocol of one of the sensitized decerebrated monkeys follows:

PROTOCOL

MONKEY 79 WEIGHT: 2.0 KG.

11-2-36 5 c.c. egg white i.v.

11-19-36

HOUR	R.P.M.	TREATMENT
9:48½		decerebrated
9:50	32	
9:55	48	
9:58	52	
9:58½	48	10 c.c. warm saline i.v.
10:01	46	
10:03	48	
10:05	48	
10:07	44	
10:08½	40	
10:09½	60	10 c.c. warm egg white i.v.
10:10½	90	
10:11	*	
10:13	†	
10:14	7 gasps	
10:15½	dead	

*Stopped breathing, slow flexion contraction of right arm and leg.
†Breathing again with squealing, gasping convulsively.

The results outlined above are incorporated in Table I.

TABLE I. REMOVAL OF CEREBRUM FROM EGG WHITE SENSITIVE MONKEYS

NO.	EW* C.C. I.V.	INTERVAL IN WEEKS	DECEREBRATED A.M.	SALINE 10 C.C. I.V. A.M.	EW 8-10 C.C. I.V. A.M.	DEATH IN MINUTES AFTER INJECTION
76	5.0	2	10:25		10:40	1
81	5.0	2	11:26	11:31	11:33	†
79	5.0	2½	9:48	9:58	10:09	6
93	2.5	3	9:49		10:03	4
			Sensitized Controls			
80	5	2	-		10:51	4
92	2	3	-		1:20	2
			Unsensitized Controls			
89	-		10:39	10:49	11:01	56
96	-		10:44	11:03	11:13	73

*EW, egg white (undiluted).
I.V., intravenous injection.
†Ten seconds.

SUMMARY

1. Decerebration in 4 egg white sensitive monkeys failed to prevent fatal anaphylaxis within six minutes of the injection of the test antigen.

2. Two unsensitized decerebrated monkeys remained unaffected by the same amount of antigen and survived for one hour after injection.

3. Two sensitized control animals died of anaphylactic shock within four minutes of the injection of the test antigen.

REFERENCES

1. Kopeloff, N., Davidoff, L. M., and Kopeloff, L. M.: General and Cerebral Anaphylaxis in the Monkey (Macacus rhesus), J. Immunol. **30**: 477, 1936.
2. Lumière, A., and Couturier, H.: Sur le rôle des centres nerveux dans le chocs anaphylactiques, Arch. internat. d. pharmaco dynamie et d. therap. **31**: 265, 1926.
3. Pearce, R. M., and Eisenbrey, A. B.: The Physiology of Anaphylactic "Shock" in the Dog, J. Infect. Dis. **7**: 565, 1910.
4. Schürer, J., and Strasmann, R.: Zur Physiologie des anaphylaktischen Shocks, Ztschr. f. Immunitätsforsch. u. exper. Therap. **12**: 143, 1912.
5. Zunz, E., and La Barre, J.: Sur la persistance des phénomènes de choc anaphylactique chez le cobaye et chez le rat décérébrés, Compt. rend. Soc. de biol. **95**: 858, 1926.

BLOOD STUDIES ON NORMAL AND TRICHINIZED WHITE RABBITS*

W. W. Wantland, Evanston, Ill.

IN THE parasitic disease, trichiniasis, where the causative organism spends a part of its life cycle in the blood stream, it seems logical that changes might be manifested in this fluid tissue as a result of infection. It has been quite definitely established that trichiniasis in man and in experimental animals gives rise to a pronounced leucocytosis and generally a marked eosinophilia, which may or may not be proportional to the degree of infection, and which may subside early in some cases but persist for months in others. Considerable variation occurs in degree of eosinophilia and length of time it persists. Cases with none, or with only very late eosinophilia, but with general leucocytosis are cited by Conner (1929) as an atypical form of trichiniasis. It is probable that a very early eosinophilia occurred in these cases followed by an aneosinophilia which sometimes occurs in helminth infections following a hypereosinophilia. It is more often the rule, however, in trichiniasis for eosinophilia to persist for some weeks. Innumerable case studies have been reported in the literature with regard to degree and persistence of leucocytosis as a result of trichina invasion.

In experimental work on trichinized monkeys, McCoy (1932) was unable to demonstrate a marked eosinophilia. This is very difficult to explain since a hypereosinophilia is typical in most animals infected with trichina in the laboratory. That the percentage of eosinophiles is not necessarily an index of the severity of the infection has been repeatedly shown in both experimental animals and in man. Opie (1904) demonstrated collections of eosinophiles in the lungs of guinea pigs early in the course of the transfer period. Later the eosinophilia decreased markedly, and upon completion of encapsulation disappeared. In the pig, eosinophilia occurs early and regularly and according to Maass (1933) is in direct proportion to the number of worms

*From the Department of Zoology, Northwestern University.
Received for publication, March 16, 1937.

ingested. The relation of eosinophile cells to animal parasites is very imperfectly understood. Banerji Nabajiban (1933) induced relative eosinophilia in rabbits by increasing the carbon dioxide tension in the blood. This work indicates that an alteration in the acid-base equilibrium incites eosinophilia.

Observations of the blood picture in trichiniasis have been largely confined to studies of white cell variations. It is interesting to note in this connection that a decided seasonal variation was observed in the number of red and white cells and in percentage of hemoglobin of normal rabbits by Pearce and Casey (1930). These fluctuations, however, were not found to parallel each other and no specific relationships were suggested by the investigators. These workers give the following averages of cell counts and hemoglobin determinations on 174 normal rabbits from Oct. 20, 1927, to Nov. 21, 1928: Erythrocytes, 5,198,000± 12,700; leucocytes, 9,562± 59; hemoglobin, 63 per cent ± 2 per cent. A range taken from counts on normal rabbits made by three groups of investigators: Bushnell and Bangs (1926), Scott and Simon (1924), and Pearce and Casey (1930) shows the following differential white cell percentages: neutrophiles, 39 to 45 per cent; eosinophiles, 1 to 3 per cent; basophiles, 1 to 10 per cent; lymphocytes, 31 to 56 per cent; monocytes, 0 to 5 per cent. The present investigation was undertaken with the purpose of making a fairly exhaustive study of the blood of trichinized rabbits to determine deviations from the normal.

BLOOD STUDIES AND METHODS EMPLOYED

The following blood studies were made on a series of twelve white rabbits:

1. *Erythrocyte and Leucocyte Counts* (Hemacytometer, Improved Newbauer ruling).

2. *Hemoglobin Estimation* (Newcomer method).

3. *Specific Gravity* (chloroform and benzene mix).—A medium sized drop of blood was suspended in a mixture of chloroform and benzene. The specific gravity of this mixture was adjusted to a point where the drop of blood remained suspended half way in the column of the mixture. The specific gravity of the mixture was then determined by means of an hydrometer.

4. *Clotting Time* (Sabrazes method).—A piece of glass tubing, 5 to 6 cm. in length, 1 mm. bore, was filled with blood by capillary attraction. The filled tube was held within the hand, and at intervals of fifteen to twenty seconds a small section was scratched with a file and broken off. Coagulation was interpreted to be complete when a worm-like coagulum was obtained at the time of the break and threads of fibrin could be seen connecting the broken ends of the tubing.

5. *Fragility Test of Erythrocytes.*—For this test a series of eight test tubes containing the following percentages of NaCl were employed: 0.220, 0.280, 0.340, 0.400, 0.460, 0.521, 0.581, 0.646. A vein of a rabbit's ear was punctured and from 20 to 40 c.mm. of blood were introduced by means of a blood pipette (capacity 5,020 c.mm.) in to each of the eight concentrations. The tubes were allowed to stand at room temperature for forty-five minutes. Hemolysis was regarded as complete when little or no sediment was visible and the solution appeared a clear transparent red color.

6. *Measurement of Erythrocytes.*—Smears were made from an ear puncture and the diameters of 100 red cells were measured under high power (280 magnification). These smears were later stained with Wright's blood stain to determine the absence or presence of eosinophilia. Composite graphs (Charts 1 and 2) indicate the averages of the measurements of the red cells of the twelve rabbits before and after infection. The average arithmetical mean, the average mode and the average mean deviation are also shown. The twelve

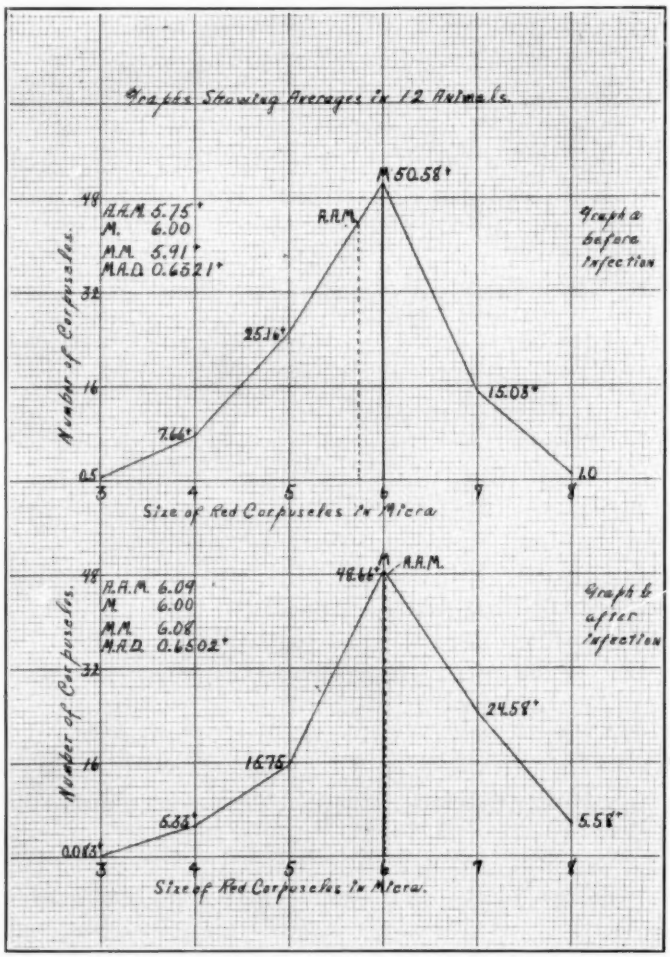


Chart 1

Chart 2

animals studied in this series of experiments were given from 5,000 to 10,000 trichinae larvae each by means of a mouth syringe. The larvae were obtained by artificial digestion of infested rat and rabbit muscle. The animal was killed, the hair removed and pieces of muscle ground in a meat grinder. This material was then placed in 250 c.c. Erlenmeyer flasks containing about 100 c.c. of artificial gastric juice (0.7 gm. pepsin plus 0.5 c.c. c.p. HCl to 100 c.c. distilled water) and allowed to digest with occasional shaking in a 40° C. water-bath from three to four hours. The contents of the flasks were then strained and approximate counts made of the free larvae in each of 5 c.c. of the undiluted

liquid containing the organisms. The number of larvae given an animal was then approximated by multiplying the average number in 1 c.c. (undiluted) by the total volume of the material administered (25 to 50 c.c.). Carrot water and dextrose were added in most cases to facilitate deglutition.

The studies on rabbits before infection with *T. spiralis* were made over periods ranging from one to seven and one-half months. After infection the various blood observations were made over a period of from one and one-half to seven and one-half months.

DISCUSSION OF FINDINGS AND CONCLUSIONS

It was shown in this series of blood studies of normal and trichinized rabbits that considerable fluctuation occurred in all factors studied except the osmotic resistance of the erythrocytes. This latter factor remained nearly constant with only an occasional fluctuation in both normal and infected animals. Slight hemolysis was observed rarely in a solution of 0.460 per cent NaCl. This concentration, in most of the tests, was sufficient to prevent laking.

In all of the animals studied from one to five observations were made per month. The fluctuations observed, especially in the studies on normal animals (before infection with *T. spiralis*) are difficult to explain. It has been repeatedly shown by investigators in physiologic and immunologic work on rabbits that even a slight deviation from the regular procedure of puncture, etc., will often result in a sudden temporary polycythemia due very probably to splenic contraction. Increased functional demands, when they occur, may give rise to such a condition. The utmost precaution was observed in this series of experiments to carry out routine examinations and observations as nearly as possible under the same conditions.

The red cell count of the normal rabbits (i.e., before infection) was found to range from 4,000,000 to 6,000,000 per c.mm. Temporary hyperglobulia (specific cause undetermined) was observed in six of the animals studied. This sudden and temporary increase in the number of red cells appeared to have no particular influence or definite relationship to the other factors observed on the same date. It might be expected that an increase in red cells would give rise to an increased hemoglobin percentage and consequently an increase in specific gravity; yet this was not found in most instances where a temporary polycythemia was observed. The fluctuation of the clotting time (2– to 2+ minutes) was noticeable but neither delayed nor accelerated coagulation occurred in any definite relationship with other factors observed.

The size of the red blood corpuscles in normal rabbits varied from 0.003 mm. to 0.008 mm. in diameter. In the hundred cells measured in each case, a diameter of 0.006 mm. was found to be a constant mode (size of greatest frequency), except in the case of one rabbit in which the red cells showed a mode of 0.005 mm. The arithmetical mean varied somewhat above or below the mode size in the different animals but the average arithmetical mean (0.00575+ mm.) was somewhat lower than the average mode (0.00591+ mm.) (see Chart 1). The mean deviation of the size of the erythrocytes from the arithmetical mean was found to vary, though not markedly, in the different animals. The average mean deviation (0.0006321+ mm., see Chart 1) definitely

expresses as a criterion of variation the variability of the individual erythrocytes in all of the normal animals, from the average arithmetical mean (0.00575+ mm.).

Following infection with *T. spiralis* a definite erythrocytosis (from 6,000,000+ to 8,000,000+) was observed in seven of the twelve rabbits infected. This increase in the number of red cells reached its highest point usually before the ninth week following infection. Only one case was observed in which the peak of the erythrocytosis occurred as late as the ninth week after infection. The number of red cells per cubic millimeter of blood, in this case, was found to be 8,540,000 which was the highest count observed in the whole series.

In all animals in which a polycythemia was observed after infection, a rise in percentage of hemoglobin was noted, roughly paralleling the increase of red cells. The highest readings of hemoglobin obtained (126.59 per cent in three animals) revealed a color index of more than 1.0 in only one case. In two other animals in which the hemoglobin reading was not so high and the red cell number sufficiently low, a color index of 1.0+ was also revealed. A color index of less than 1.0 was observed throughout the series with the exception of the above cases after infection and two cases before infection. Since the color index in the majority of observations of both normal and infected animals was less than 1.0, this condition cannot be looked upon as a result of infection with *T. spiralis*.

The specific gravity of the blood almost always increased with a rise in hemoglobin and an increase in number of erythrocytes.

Marked leucocytosis (12,000 to 23,200) was the rule following infection and was observed as early as the second week following the ingestion of trichinae larvae. Eosinophilia was observed in all infected animals and varied from 25 to 79 per cent in different animals during the third week following infection. The symptoms of trichiniasis (dyspnea, difficulty in mastication, edema, etc.) were decidedly more severe in rabbits in which the increase of eosinophiles was the greatest. Eosinophilia (as high as 50 per cent) persisted in one rabbit for as long as seven months and this condition was still present in others from two to four months after infection. The condition known as aneosinophilia, which sometimes follows hypereosinophilia in worm infections, was not observed in any of the infected animals.

The clotting time fluctuated after, as well as before, infection. From three to five weeks following infection a noticeable decrease (from 2+ to 2– minutes) in this factor was observed. This finding at this time during the blood phase or early muscle phase of the disease may have been due to an increase of calcium in the blood, mobilized as a protective measure against small hemorrhages caused by the passing of trichinae larvae from the capillaries to the striped muscle.

Occasional slight changes in the osmotic resistance of the erythrocytes of both normal and infected animals were observed. The same changes in the permeability of the red cell membrane are known to occur during the general metabolism of the body, as occur in other cell membranes. The properties of the cell membrane are not fixed but are modified from time to time by the many chemical processes inside and by influences outside.

Measurements of the red blood cells made from thirteen days to three months and three days after infection showed a definite macrocytosis in ten of the twelve infected rabbits. In the remaining two animals a microcytosis was noted after infection. The degree of macrocytosis was found to vary in the ten different animals as did the degree of microcytosis in the other two cases. When the results observed in all twelve infected animals are averaged and viewed compositely (see Charts 1 and 2) a definite and marked increase in size of the erythrocytes is noted (A.A.M.). The average arithmetical mean as shown in these curves increased from 0.00575+ mm. to 0.00609 mm. after infection. The true modes (M) of both Charts 1 and 2 remain the same (6), but the mean of all separate modes (arithmetical average M.M.) increased from 0.00591+ mm. to 0.00608 mm. after infection. The average mean deviation (M.A.D.) showed only a slight increase from 0.0006321+ mm. to 0.0006502+ mm. after infection. The occurrence of a definite macrocytosis usually less than three weeks after infection with *T. spiralis* in the majority of rabbits studied with regard to this factor would seem to indicate a definite reaction of the erythrocytes to the parasite or toxic substances. The cause of the macrocytotic condition is at best speculative. It may be that toxic products, produced by the larvae in the blood stream and in the early muscle phase when the muscle tissue undergoes degeneration, alter the permeability of the erythrocyte membrane in such a manner as to allow increased diffusion into the corpuscle of certain salts, water, etc., but prevent to a limited degree outward diffusion into the plasma. Such a condition would undoubtedly result in swelling of the red cells. Reactions of this nature might well be brought about, especially if the diffusing substance is largely water, through a slight lowering of the osmotic pressure of the plasma bathing the cells, by the toxins produced by the larvae or other factors. The law of diffusion would then operate, diffusion taking place from regions of greater to lesser concentrations. The cell contents would here be considered as hypotonic (water diluted by constituents) and the surrounding plasma as hypertonic, the more concentrated of the two regions with regard to diffusible substance.

Such a change in osmotic pressure of the plasma or content of the erythrocyte would undoubtedly be extremely minute. However, even if the change were more marked, it would not be likely that the difference in osmotic resistance of the cell could be detected by fragility tests generally employed. A much more delicately adjusted set of concentrations would be necessary and even then the conditions in vivo would very probably be only roughly approximated. Further experimentation is necessary in order that the nature of the change here encountered be understood.

SUMMARY

Blood studies on a series of twelve trichinized rabbits revealed that a polycythemia occurring as early as the second week after infection, closely paralleled by an increase in hemoglobin percentage and a rise in specific gravity, occurred in seven of the twelve animals following infection with *Trichinella spiralis*.

Hypereosinophilia was definitely associated with marked symptoms of trichiniasis. A noticeable decrease in clotting time, three to five weeks following infection, and a definite erythrocytic macrocytosis (observed in most cases less than three weeks after infection) seemed indicative of trichina infection in the white rabbit.

The production of toxins in the intestinal, blood and muscle phases of the disease very probably is one of the main factors concerned in the disturbance of the normal blood picture.

REFERENCES

Badel, A.: Zur Frage der Häufigkeit der Trichinose beim Menschen, Wien. klin. Wchnschr. **46**: 302, 1933.

Bushnell, L. D., and Bangs, E. F.: A Study of the Variation in Number of Blood Cells of Normal Rabbits, J. Infect. Dis. **39**: 291, 1926.

Conner, L. A.: Atypical Forms of Trichinosis, Ann. Int. Med. **3**: 353, 1929.

Flury, F.: Beiträge zur Chemie und Toxikologie der Trichinen, Arch. f. Exp. Path. u. Pharmakol. **73**: 164, 1913.

Maass, Z. T.: Ueber Eosinophilie im Schweineblut bei Trichinose, Zentralbl. f. Bakt. (Abt. 1) **129**: 29, 1933.

McCoy, O. R.: Experimental Infections in Monkeys, Proc. Soc. Exper. Biol. & Med. **30**: 85, 1932.

Nabajiban, Banerji: The Induction of Eosinophilia in Normal Animals, Am. J. M. Sc. **186** (5): 689, 1933.

Opie, E. L.: Relation of Eosinophil Cells to an Animal Parasite, Am. J. M. Sc. **127**: 477, 1904.

Pearce, L., and Casey, A. E.: Studies on the Blood Cytology of the Rabbit, J. Exper. Med. **51**: 83, 1930.

Pewsner, M.: Zur Charackteristik einer Trichinellen Epidemie, Arch. f. Schiffs- u. Tropen-Hyg. **36**: 88, 1932.

Scott, J. M., and Simon, C. E.: Experimental measles. I. The Thermic and Leucocytic Response of the Rabbit to Inoculation with the Virus of Measles, and Their Value as Criteria of Infection, Am. J. Hyg. **4**: 559, 1924.

MULTIPLE SEROSITIS, "KELLY" PERICARDITIC PSEUDOCIRRHOSIS OF THE LIVER, "PICK"

THOMAS L. RAMSEY, M.D., TOLEDO, OHIO

THIS condition frequently overlooked in many modern textbooks on medicine with its wide variation in pathologic findings and with its varied symptomatology is much more frequent in its incidence than suspected. The first article on this subject was that by van Deen[1] in 1847. Subsequent articles by various authors are cited by Kelly[2] in his monograph of the subject, *Multiple Serositis*, which appeared in January, 1903.

In 1885 Curschmann[3] described his now well known "Zuckergussleber," or chronic hyperplastic perihepatitis. This condition was also apparently well known to a number of the earlier writers, the first of whom was Rokitansky,[4] who described it in his Handbook on special pathologic anatomy in 1842.

Kelly's monograph includes all of the cases up to 1903 including Pick's,[5] who described the multiple involvement of serous membrane inflammations calling attention to the symptom complex of chronic adhesive pericarditis associated with pseudocirrhosis of the liver and recurrent ascites. Pick's first article was published in 1896,[6] other papers by Pick were published in 1899 and 1900.[7]

The condition was described as chronic perihepatitis and mediastinopericarditis by Osler,[8] in his *Practice of Medicine* in 1895, with a subsequent article on the subject in 1896.[9] A good description is also present in Osler's *Modern Medicine* edited by McCrae;[10] the text was originally written by Kelly and later revised by Lyon.

Other writers on this subject have given rise to quite a varied nomenclature. Some of the terms used to describe this and similar conditions are as follows. Italian writers speak of it as polyserositis tuberculosa; Concato's disease; Concato's original article, poliorromennite scrofolosa appeared in the literature in 1881;[11] the French have described it as polyvisceritis and as polyorrhomenitis; Nicholls[12] wrote of it as chronic progressive hyaloserositis; Dawd[13] described it as chronic plastic peritonitis; Osler as proliferative peritonitis.

This multiplicity of terms for what appears to be one and the same condition but showing a variety of etiologic factors, symptoms, and areas of involvement and even with some variation in the pathologic changes is certainly confusing to say the least and some definite nomenclature for the condition should be established. It appears that the name used by Kelly, multiple serositis, is being more generally accepted by recent writers and seems to fulfill the necessary requirements.

As to its etiology, the disease is about equally common in both sexes and has been described as occurring at all ages but commonly seen in the second

*From the Department of Pathology, St. Vincents Hospital.
Received for publication, March 15, 1937.

and fifth decades; the exciting causes are not definitely understood but they are probably toxic in nature, the source of which is not known. The condition has been associated with arteriosclerosis and chronic interstitial nephritis but it is more probable that these conditions are due to the same etiologic factors as the multiple involvement of the serous membranes. This multiple involvement suggests a rather widespread factor suggesting bacterial toxins acting over a rather long period of time and of a low grade type. As has been mentioned by Concato, a number of these cases are due to tuberculosis and it is important to bear in mind that a number of cases apparently nontuberculous are in reality due to the tubercle bacillus and that characteristic tuberculous lesions may be found if a very careful and thorough search is made. It has been suggested that a different form of tubercle bacillus may be the causative agent such as the avian type, Cantu cited by Hager.[14]

Pathologic Anatomy.—The site of origin of the serous membrane involvement is apparently different in the cases observed and the pathology varies according to the location in which the disease process has its incipiency. De Renzi[15] believed the peritoneum to be usually involved first, then the right pleura and then the pericardium and if the right pleura was involved first, then that the disease extended to the peritoneum and thence to the left pleura and the pericardium. Whether the lesion begins above or below the diaphragm, the ultimate lesions are generally the same and the condition is essentially a chronic multiple serositis.

It generally pursues a remarkably slow and insidious course giving rise to exudation of large quantities of serofibrinous fluid, the fluid portion of the exudate is generally reabsorbed, leaving the fibrinous substance deposited upon the involved viscera. This fibrinous deposit is then invaded by leucocytes, mast cells, and fibroblasts; blood vessels are scant, and there is a tendency toward lamellar formation.

This peculiar overgrowth of fibrous tissue may become hyalinized, giving the deposit a glistening appearance, it may even resemble a cartilaginous substance and the deposit of lime salts may give rise to the formation of bone-like plaques; case cited by Stone.[16] Dense adhesions to the surrounding tissue may undergo cicatricial contraction so that distortion of the involved viscera may occur.

The association of pericarditis with perihepatitis is the common finding and develops into adhesive pericarditis with the gradual interference of cardiac function and the development of the lesions associated with it. The ascites which develops is generally abundant and the fluid generally rapidly reappears after tapping. This fluid is generally of a yellow, or amber tint showing albumin and a specific gravity of 1.015 or higher. Fluid also may develop in the pleural and pericardial sacs but this is generally reabsorbed, leaving the characteristic fibrinous deposit with the ultimate development of dense adhesions.

The pericardial involvement may vary in extent but is usually progressive from a few adhesions to complete obliteration of the sac and may extend to the surrounding tissues. The heart is usually hypertrophied due to myocardial

damage or vascular defect. Smith[17] found the incidence of adherent pericarditis to be 62 in a series of 3,052 necropsies.

The omentum may be the site of considerable involvement and may be so thickened and matted together with segments of the intestines that abdominal neoplasm may be suspected. Dawd reported a case with autopsy findings which showed the incasement of the intestines by dense fibrinous membrane and cites a similar case by Porter[18] and one by Muir[19] where the peritoneum of the intestines was markedly thickened and contracted, producing what appeared to be extraordinary shortness of the small intestine in Muir's case and producing intestinal obstruction in Porter's case. While Dawd's case cannot be classified as Pick's disease or as Curschmann's disease, it might fall under the heading of chronic multiple serositis of Kelly or of Osler's proliferative peritonitis.

Rolleston[20] described a definite microscopic difference between the deposits on the serous surfaces, especially the liver in Pick's disease and in Curschmann's disease. In Pick's disease the deposit is under the capsule and in Curschmann's disease the overgrowth is outside the capsule. Kelly thinks these conditions are similar or at least merge into each other in certain instances. Gill and Graig[21] discussed a difference between Pick's disease and Concato's disease and especially the difference between Curschmann's iced liver and Concato's disease.

Hamman[22] in an article on serous membrane tuberculosis reported two cases and called attention to serous membrane tuberculosis as a manifestation of the course of tuberculosis infection and that tuberculous polyserositis had a close relationship to the group of cases described in the literature as sugar iced liver of Curschmann and the pericarditic pseudocirrhosis of Pick.

Evans[23] reported a case which he designated as polyserositis and quotes considerably from Kelly's article on this subject. His case presented widespread disease of the serous membranes but did not present the typical findings of either Pick or Curschmann, and ascites was not a factor nor was adherent pericardium present.

Lamb[24] reported two cases, one of which was a typical Curschmann's with the liver showing the sugar-iced coating. The other case, a female, showed multiple serous membrane involvement and at autopsy showed typical tuberculous lesions in the spleen and in a caseous lymph node.

Kelly attempted to classify cases into three main groups.

1. Those with pericarditis but without symptoms and usually only recognized at autopsy.

2. Those with pericarditis showing early or late cardiac symptoms and findings.

3. Those with recognizable cardiac findings and symptoms associated with ascites but with little or no edema of the legs. In this third group the diagnosis of cirrhosis of the liver was usually made and the pericarditis and other morbid changes such as pleuritis with or without effusion, perihepatitis and peritonitis were generally overlooked. The signs of failing cardiac muscle, palpitation, dyspnea, cough, cyanosis, generalized edema generally called attention to the pericardial involvement.

Symptomatology.—The symptoms as may well be understood from the variation in the pathology are quite variable and the disease may remain latent for many years and may be entirely unsuspected during life. In those cases with manifest symptoms there is generally abdominal discomfort, often pain in the epigastrium, gradual distention of the abdomen with fluid so that it may attain considerable size and with no or very late edema developing in the legs. There may be slight or temporary jaundice. The clinical picture may be benign but varies somewhat depending upon the site of origin.

The development of the ascites may be the first indication of the condition, or the failing heart may be the factor which calls attention to the association of the heart condition with some other complicating factor. The heart symptoms may be those of myocardial involvement with coexisting valvular changes, palpitation, dyspnea, cough, cyanosis and edema. The pain in some cases may be anginoid in character.

Other symptoms due to circulatory disturbance may occur, such as general anasarca, also arteriosclerotic involvement with the symptoms that go with it. The gastrointestinal functions are generally well performed and emaciation is usually long postponed. The failing heart and its consequences or some intercurrent complication generally terminates the picture and death ensues.

The diagnosis of this condition is often difficult but the presence of a recurrent ascites in a person in otherwise fairly good health with the ability to be up and about between tappings, together with the character of the ascitic fluid and generally showing some pleural or pericardial involvement with thickening of these membranes or effusions into the cavities and the slow protracted and intermittent course should call our attention to the possibility of multiple serositis. Hepatic cirrhosis, syphilis, tuberculous peritonitis, and malignant disease must be ruled out.

The prognosis is bad as to eventual recovery but remissions may occur. In about 70 per cent the duration of life is two years or more; in about 50 per cent, four years or more; this of course varies with the general health of the patient and with the amount of pericardial involvement.

Treatment.—The treatment has been almost wholly symptomatic but more recently operative procedures have been used with success. In those cases where chronic constrictive pericarditis is present with symptoms of circulatory failure, resection of portions of the adherent pericardium has been performed with definite relief over varying periods of time. Beck and Cushing,[25] reported 9 cases, 6 of the patients were operated upon for relief of this condition. Three were living apparently receiving lasting benefits; one had just recently been operated upon and showed improvement in the circulation. They stressed the importance of a correct diagnosis and called attention to other previous articles on surgery of the heart for adhesive pericarditis.

An editorial[26] in the *Journal of the American Medical Association* gave an excellent review of the development of our knowledge of constrictive pericarditis and called attention to the writings of White on the treatment by pericardial resection. The editors call attention to the fact that there have been numbers of operative cures in Germany and the United States and that it is important that this disease be recognized by the medical profession.

I wish to report a case observed by me together with laboratory data and autopsy findings.

Case Report.—J. P., a male negro, aged twenty-one years, weight on admission 123 pounds. Entered the hospital following repeated attacks of tonsillitis and rheumatic fever with a provisional diagnosis of mitral insufficiency. Pain in the cardiac region with dyspnea and some edema of the ankles was present upon admission. The chest showed decreased expansion and some dullness over both bases. A marked systolic and diastolic murmur was present at the apex and a blowing systolic murmur at the base over the aortic area. The cardiac impulse was diffuse over the left side of the chest. The pulse rate was regular but

Fig. 1.—Photograph of heart, weight 885 gm., showing thickened and adherent pericardium and marked thickening of ventricular walls.

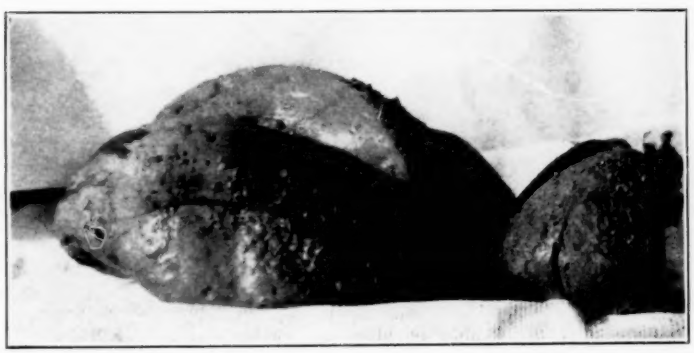


Fig. 2.—Photograph of liver and spleen showing the thickened capsules and deposits of fibrinous exudate upon the thickened peritoneal surfaces.

varied from 80 to 100. The blood pressure was 100/40. Electrocardiographic examination gave evidence of myocardial damage.

Patient left the hospital but soon returned showing signs of cardiac failure, ascites with edema of limbs and ankles and all previous findings somewhat intensified.

The impression of this case at the time was that of a rheumatic endocarditis with valvular lesions and myocardial failure. An x-ray examination of the chest showed marked enlargement of the heart shadow in all diameters with a suggestion of pericarditis with effusion.

Paracentesis was performed removing 3,375 c.c. of straw-colored serous fluid. The patient seemed somewhat relieved but the fluid rapidly recurred. Patient did not react to medication and died about ten days after his second hospital admission. An autopsy was performed.

Autopsy Findings.—Only the positive and significant findings will be included in this report. Gross examination: The body showed evidence of loss of weight; a moderate distention of the abdomen was present. There was considerable edema present in the lower extremities. Upon opening the thorax, the lungs were found congested and edematous, rather dense adhesions were present around the apices of the lungs. The pleura was

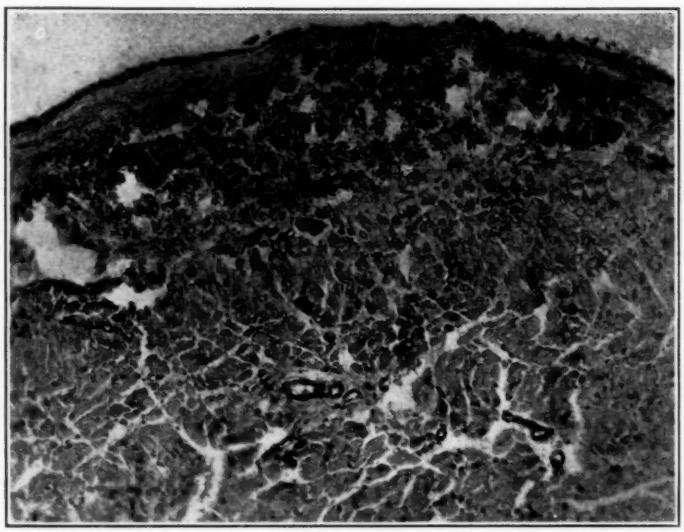


Fig. 3.—Microphotograph, through area of ventricular wall showing thickened and adherent pericardium and degenerative changes in heart muscle.

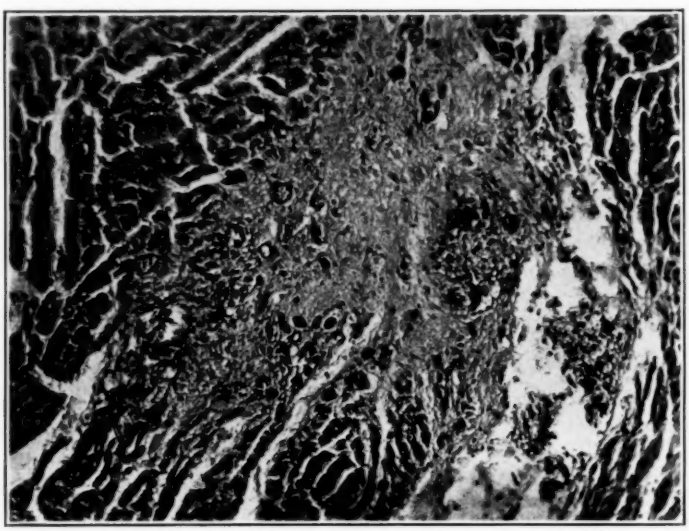


Fig. 4.—Microphotograph, typical Aschoff's node in interstitial tissue between muscle bundles in wall of left ventricle suggesting a rheumatic origin of the condition.

considerably thickened on both lungs, irregular areas having a lymphoid appearance were present. The left lung was partially collapsed and was consolidated in the lower lobe.

The heart was enlarged and the pericardium thickened and completely adherent. The heart weighed 885 gm. The left ventricular wall measured 3 cm., the right 2 cm. at the base. The tricuspid, mitral and aortic valves showed thickening of the cusps and the

valve openings were somewhat constricted. The aorta and coronary arteries showed a moderate degree of sclerosis. The abdomen contained about 2 liters of orange-colored serous fluid. The liver was about normal in size, the peritoneal surface, especially the superior surface, showed a marked nodular thickening of the capsule. The substance of the liver itself was rather soft. The spleen weighed 230 gm. and showed thickening of the capsule with a patchy grayish deposit on the peritoneal surface. The pulp was dry and firm. The other abdominal viscera including the kidneys, stomach, small and large intestines showed no remarkable pathology. The mesenteric lymph nodes were all enlarged. Sections were taken from the various viscera for microscopic examination.

Microscopic sections from the lungs, heart, liver, and spleen all showed thickening of the serous membrane due to a fibrocellular deposit on its surface with a round cell inflammatory reaction in the underlying tissues. The findings were in general similar to the case reports previously published describing this condition, and for the sake of brevity will be omitted from this article.

A summary of the autopsy findings: congestion and edema of the lungs, small area of consolidation in the lower lobe of the left lung, chronic exudative pleuritis with adhesions over and around both apices.

Chronic adhesive pericarditis with obliteration of the pericardial sac, cardiac hypertrophy and myocardial degeneration and fibrosis. Chronic endocardial inflammation with sclerosis of mitral, aortic and tricuspid valves. A moderate atherosclerosis of the aorta and coronary arteries.

Abdominal ascites, atrophic sclerosis of the liver, spleen and pancreas. Chronic inflammatory fibrosis and thickening of the capsule of the liver, perisplenitis, chronic congestion of kidneys. Fatty degeneration of the adrenal glands.

SUMMARY

This case presents the picture of multiple serositis or pericarditic pseudocirrhosis of the liver "Pick" or using the more recent term, chronic constrictive pericarditis. The extreme myocardial damage in this case may have been due to the rheumatic involvement mentioned in the history. The multiple involvement of the serous membranes had not probably progressed to such an extreme extent as is generally found in these cases, although the involvement of the pericardium was complete. The ascites was not a marked finding until cardiac failure occurred, but the character of the fluid was similar to that generally encountered in Pick's disease. There was no evidence in any of the sections that tuberculosis of the serous membranes was present.

CONCLUSION

A discussion of multiple serositis, "Kelly" or pericarditic pseudocirrhosis of the liver "Pick" together with reports and opinions of various authors on the subject has been presented. One additional case is included with a brief history, progress of the disease, and the autopsy findings with microphotographs from the tissues removed.

The references of the various authors quoted is appended.

REFERENCES

1. Van Deen, I.: Eeen geval van pericarditis chronica gecompliceerd met peritonitis chronica. N. Arch. v. bin.- en buitenl. Geneesh, Zwolle **2**: 1, 1847.
2. Kelly, A. C.: Multiple Serositis, Am. J. M. Sc. **125**: 116, 1903.
3. Curschmann, H.: Zur Differential Diagnostik der mit Ascites verbundenen Erkrankungen der Leber und des Pfortadersystems, Deutsche med. Wchnschr. **10**: 564, 1884.

4. Rokitansky, Carl: Handbuch der speciellen patholo-gischen Anatomie, **2**: 317, 1842.
5. Pick, F.: Ueber Chronische, unter dem Bilde der Lebercirrhose verlaufende Pericarditis (pericarditische Pseudolebercirrhose), Ztsche. f. klin. med. **29**: 385, 1896.
6. Pick, F.: Ueber pericarditische Pseudo Lebercirrhose, Verhandl. d. Cong. f. innere Med; Wiesb, **17**: 588, 1899.
7. Pick, F.: Erwiderung auf Dr. Eisenmenger's Aufatz, "Ueber die Sogenannte Pseudolebercirrhose," Wien. klin. Wchnschr. **13**: 324. 1900.
8. Osler, William: Osler's Practice of Medicine, New York, 1896, D. Appleton-Century Co., p. 625.
9. Osler, William: On the Association of Enormous Heart Hypertrophy, Chronic Proliferative Peritonitis, and Recurring Ascites, with Adherent Pericardium, Arch. Pediat. **13**: 3, 1896.
10. Osler, William: Osler's Modern Medicine; McCrae. Article by Kelly, revised by Lyon. **3**: New York, 1926, Lea and Febiger, p. 783.
11. Concato, L.: Sulla poliorromennite scrofolosa o tisi delle sierose. Gior. internaz. d. sc. med., Napoli **3**: 1037, 1881.
12. Nicholls, A. G.: A Somewhat Rare Form of Chronic Inflammation of the Serous Membranes, Multiple Progressive Hyaloserositis; Studies From the Royal Victoria Hospital, Montreal **1**: No. 3, 1902.
13. Dawd, C. N.: The Relationship Between Certain Forms of Intestinal Obstruction, Chronic Peritonitis and Chronic Multiple Serositis, Ann. Surg. **77**: 423, 1923.
14. Cantu: Cited by Hager, J: Ueber polyserositis, Festschift zur Feier des 50 jahrigen Gestehens der medicinischen, Gesellschaft zu Magdeburg, p. 39, 1898.
15. De Renzi: Cited by Hager, J.: Gesellschaft zu Magdeburg, p. 39, 1898.
16. Stone, W. J.: Adherent Pericardium with Calcification, Am. Heart J. **1**: 434, 1926.
17. Smith, W. H.: Chronic Adherent Pericarditic, J. A. M. A. **61**: 739, 1913.
18. Porter, M. F.: Chronic Peritonitis with Complete Obstruction, Caused by Numerous Constrictions, of a Previously Undescribed Character, Thruout the Intestine, Trans. Sect. on Surg. & Anat. A. M. A., p. 202, 1908.
19. Muir, J. C.: A case of extraordinary shortness of the small intestine. British Med. Jour. V. II; p. 73, 1914.
20. Rolleston, H. D.: Pericarditic Pseudo-cirrhosis of the Liver. Diseases of the Liver, Gall-bladder and Bile Ducts, New York, 1912, The Macmillan Company, p. 96.
21. Gill, A. W., and Craig, T.: Pericarditic Pseudo-cirrhosis of the Liver, or Pick's Disease, Brit. M. J. **5**: 241, 1929.
22. Hamman, Louis: Serous Membrane Tuberculosis, Med. Clin. N. A. **2**: 1747, 1919.
23. Evans, G. H.: Multiple Serositis, Report of a Case with Autopsy Findings; Discussion of its Classification, Journal **155**: 553, 1918.
24. Lamb, A. R.: Multiple Serositis, Med. Clin. N. A. **4**: 1329, 1921.
25. Beck and Cushing: Chronic Intrapericardial Pressure. A Type of Circulatory Failure that Frequently can be Cured by Operation, J. A. M. A. **102**: 1543, 1934.
26. Editorial: J. A. M. A. **105**: 1120, 1935.

IRON METABOLISM IN HEMOCHROMATOSIS*

W. M. Fowler, M.D., and Adelaide P. Barer, Ph.D., Iowa City, Ia.

THERE are many excellent clinical and pathologic studies of hemochromatosis and the end-results of this syndrome, consisting of pigmentation and fibrosis in various organs, are well recognized. Analyses of the liver, spleen, lymph nodes, and pancreas as well as other organs have shown an iron content which is greatly in excess of the normal,[1-3] but the pathogenesis and the origin of these excessive amounts of iron have not been adequately explained. There is no evidence of excessive hemolysis of the erythrocytes inasmuch as jaundice, increased bilirubinemia and significant anemia are not present in the typical case. Excessive hemolysis, produced experimentally in animals, does not lead to comparable pathologic lesions,[1] and chronic hemolytic anemia, even though the hemolysis has been present for many years and has resulted in the deposit of large amounts of hemosiderin, does not simulate hemochromatosis either clinically or pathologically. Mallory[4, 5] has presented experimental evidence that chronic poisoning with copper leads to pathologic changes comparable to hemochromatosis and has shown that a group of patients with this syndrome were all unduly exposed to copper.[6] This explanation has not been universally accepted. Many other suggestions have been advanced and discarded and the discussion as to whether the cirrhosis of the liver is the cause or the result of the pigment deposits continues. Cases have been reported[7, 8] in which there is an obvious endocrine dyscrasia, usually a hypogonadism or infantilism, but this appears to be secondary to the pigmentation and fibrosis rather than of etiologic significance.

Muir and Dunn[2] suggest that the cells of the liver and other organs have acquired an unusual affinity for iron and the metal becomes fixed in their protoplasm, first in a soluble form and later as granules of hemosiderin. Attempts have been made to show an abnormal retention or a diminished excretion of iron in these patients. Howard and Stevens,[9] in one case which was studied late in its course, found a slight but definite retention of the dietary iron without evidence of undue destruction of the erythrocytes. McClure[10] found that 20 per cent of 240 mg. of ingested iron was not recovered in the feces and that no iron was present in the urine. He believed that these findings indicated an abnormal iron retention. Garrod and his coworkers[11] believed that there was a failure to excrete iron by the usual channels in hemochromatosis since they were unable to find any iron in the urine, bile, or feces of such a patient. Dry[12] has recently reviewed the literature on hemochromatosis and iron metabolism and in a metabolic study on one patient found a positive iron balance in three of five balance periods. In the first three day period 7.66 mg. of iron were retained from an intake of 20.1 mg.

*From the Department of Internal Medicine, Medical School, State University of Iowa.
Received for publication, April 12, 1937.
This investigation was supported in part by a grant from the Eli Lilly Company.

and in the fourth period 252 mg. were retained from a total intake of 967 mg. The patient retained iron in a subsequent period but the amount was not determined. These investigations have shown that iron is retained by patients with hemochromatosis. They have not demonstrated, however, that this iron retention is greater than occurs in normal individuals.

Iron-balance studies were performed on four patients who presented the characteristic features of hemochromatosis and, as a control, similar studies were performed on two patients with uncomplicated diabetes mellitus who were in the same age group and whose diabetes was of approximately the same severity as that in the patients with hemochromatosis. The essential clinical features of these patients are given in Table I. Case 3 was in a most

TABLE I

CASE	SEX	AGE	DIAGNOSIS	CLINICAL FEATURES
1	M	68	Diabetes mellitus	Symptoms of diabetes for thirteen years. Controlled by diet without insulin. Admitted with infected finger. Blood sugar 376. Gastric acidity normal.
2	M	51	Diabetes mellitus	Symptoms of diabetes for three years. Never used insulin. Blood sugar 447. Gastric acidity normal.
3	M	50	Hemochromatosis	Weakness, loss of weight and strength for four months. Emaciated, dilated, and tortuous abdominal veins. Liver markedly enlarged. Skin dark, dusky brown. Blood sugar 367. Gastric acidity—no free HCl. Wassermann 4 plus. Autopsy.
4	M	53	Hemochromatosis Multiple neuritis	Weakness and loss of weight. Symptoms of peripheral neuritis. Skin—brownish pigmentation. Liver slightly enlarged. Diminished glucose tolerance. Fasting blood sugar not elevated. Gastric acidity normal.
5	M	50	Hemochromatosis	Symptoms of diabetes mellitus for three months with loss of weight and strength. Skin extremely dark grayish brown. Liver enlarged. Blood sugar 337. Gastric acidity normal.
6	M	47	Hemochromatosis	Symptoms of diabetes mellitus for six months with loss of weight and strength. Liver enlarged. Deeply pigmented skin and a biopsy specimen gave a positive reaction for iron. Blood sugar 455. Gastric acidity normal.

advanced stage of the disease and died shortly after the completion of the balance studies. Necropsy revealed the characteristic features of hemochromatosis. Case 4 may be considered to be in a relatively early stage since a diminished glucose tolerance was the only manifestation of diabetes. All patients except Case 3 had a normal gastric acidity so that with this one exception the absorption of iron was not influenced by achlorhydria. In none of the patients was there an increased bilirubinemia.

The patients were placed on a diet of known iron content for a three day adjustment period before observations were begun. The subsequent balance periods were of six days duration except for Case 3 which was only four days. The iron intake from the diet and the insulin was determined for each balance period. The urine and the stool specimens for each period were collected and analyzed for their iron content. From these data were calculated the iron balance for that period and the average daily iron balance. Complete nitrogen and phosphorus balances were done in all cases. Cases 1, 2 (controls), and 6 were given 2 gm. of iron and ammonium citrates on the first day of their last balance period in order to study the iron retention from a larger intake of a

soluble iron preparation. The methods in more detail and the technical procedures were described in a previous report on iron balances.[13] Nitrogen was determined by the Kjeldahl method and phosphorus by that of Fiske and Subbarow.[14] Iron determinations were made in triplicate by the method of Reis and Chakmakjian.[15]

RESULTS

The results of the nitrogen, phosphorus, and iron balances are given in Table II and a summary of the iron balances in Table III. The results are expressed as the average daily balance for each of the six day balance periods. In the control subjects it was found that Case 1, with a daily iron intake of 13.44 mg., was in a negative balance of 5.14 mg. and 0.51 mg. per day during the two periods of observations and Case 2 showed a loss of 1.19 mg. per day during the first period but a retention of 0.21 mg. per day during the second period.

Case 3, whom we were able to study for only four days, showed a negative balance of 18.6 mg. of iron per day. Certain complicating features in this patient, however, contrive to render this an unreliable result. The patient's appetite was poor, he had a complete achlorhydria and, in addition, he continually lost small amounts of blood in the stools from bleeding esophageal varices. Although no evidence of iron retention could be detected under these circumstances the analysis of the liver, pancreas, and kidney, when obtained at necropsy, showed an increase in their iron content, Table IV.

In case 4 there was a retention of 5.42 mg. of iron per day from an intake of 13.42 mg. or a total retention of 32.52 mg. from an intake of 80.52 mg. during the six-day period. This was much higher than was found in the other cases of hemochromatosis or in the control subjects receiving a similar amount of iron. We have encountered a retention of this degree in only two subjects (Reference 16, Table VII, Cases 1 and 2) and then only when the patient was receiving a diet containing over 14 mg. of iron per day. It is unfortunate that we were able to study the patient for only one six-day balance period but inasmuch as he represented a relatively early stage of hemochromatosis, without fully developed diabetes, it suggests that there is an increased iron retention early in the course of the disease.

Case 5 was studied for three balance periods and was consistently in a negative iron balance, showing a loss of 8.35, 7.12, and 4.73 mg. of iron per day. The patient was in an advanced stage of the disease and was losing iron quite rapidly rather than retaining it. Case 6 was observed for four balance periods in which iron was obtained from the diet alone. During the first period he retained 1.94 mg. per day but for the subsequent three periods was consistently in a negative iron balance, losing 0.80, 0.45, and 4.74 mg. per day respectively and for the entire four periods, a total of twenty-four days, he was in a definitely negative balance.

Cases 1, 2 (controls), and 6 were given a single dose of 2 grams of iron and ammonium citrates with their breakfast on the first day of the final balance period in an attempt to determine whether a large amount of iron would be handled differently by a patient with hemochromatosis than by the control

TABLE II

CASE	PERIOD	DAILY NITROGEN BALANCE			DAILY PHOSPHORUS BALANCE			DAILY IRON BALANCE			ERYTHROCYTES MILLIONS PER C.C.	HEMATOCRIT PER CENT	HEMOGLOBIN GM.
		INTAKE GM.	EXCRETION GM.	BALANCE GM.	INTAKE GM.	EXCRETION GM.	BALANCE GM.	INTAKE GM.	EXCRETION GM.	BALANCE GM.			
1	I	10.97	11.15	− .18	1.19	1.23	− .04	13.44	18.58	− 5.14	4.05	37.0	11.150
	II	10.97	10.25	.72	1.19	1.46	− .27	13.44	13.95	− .51	4.18	38.0	12.510
	III*	11.00	10.54	.46	1.19	1.30	− .11	66.90	22.03	44.87	4.51	40.0	12.280
2	I	10.97	9.10	1.87	1.19	1.09	.10	13.43	14.62	− 1.19	4.55	40.0	12.910
	II	10.96	9.12	1.84	1.20	1.27	− .07	13.54	13.33	.21	4.80	42.0	12.910
	III*	11.00	9.08	1.92	1.19	1.13	.06	66.89	30.03	36.86	4.32	41.0	13.610
3	I	5.47	5.97	− .50	.79	.79*	− .00	8.59	27.19	−18.60	−	31.5	9.020
4	I	10.97	10.98	− .01	1.19	1.06	.13	13.42	8.00	5.42	4.59	45.0	11.100
5	I	10.48	12.39	−1.91	1.16	1.27	.11	12.97	21.23	− 8.35	4.71	41.0	12.170
	II	6.88	10.21	−3.33	.89	1.04	− .15	9.22	16.37	− 7.12	3.89	38.0	11.990
	III	10.55	9.36	1.19	1.20	1.09	.11	13.34	18.07	− 4.73	3.93	38.0	11.360
6	I	10.97	11.01	− .04	1.19	1.13	.06	13.43	11.49	1.94	4.73	44.0	11.290
	II	10.97	10.58	.39	1.19	.96	.23	13.43	14.23	− .80	4.98	44.0	11.290
	III	11.02	11.82	− .80	1.20	1.54	− .34	13.53	13.99	− .45	4.99	44.0	11.290
	IV	11.02	10.19	.83	1.20	1.35	− .15	13.53	18.28	− 4.74	4.56	44.0	11.290
	V*	11.05	11.97	− .92	1.20	1.74	− .54	67.01	30.46	36.55	4.49	44.0	11.380

*Two grams of iron and ammonium citrates given with breakfast on the first day of the period.

TABLE III

AVERAGE EARLY IRON RETENTION BY PERIODS—MG.

CASE	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
1	− 5.14	−0.51	44.87*		
2	− 1.19	0.21	36.86*		
3	−18.60				
4	5.42				
5	− 8.35	−7.12	−4.73		
6	1.94	−0.80	−0.45	−4.74	36.55*

*340 mg. of iron on first day of the period.

TABLE IV

ANALYSIS OF TISSUES FOR IRON. CASE 3

ORGAN	WEIGHT	IRON MG.	NITROGEN GM.	PHOSPHORUS GM.
Liver	3,030	12,613.09	74.93	7.56
Pancreas	220	86.15	3.59	0.76
Kidney	260	180.18	2.86	0.32

subjects. Case 1 shows an average daily iron retention for this period of 44.87 mg. which represents a retention of 269.22 mg. or 63 per cent of the 401.4 mg. ingested during the six-day period. Case 2 retained 36.86 mg. per day, a total of 221.16 mg. or 55 per cent of the 401.36 mg. ingested. In Case 6 the average daily retention was 36.55 mg. of iron, the total retention for the six-day period being 219.3 mg. or 54 per cent of the 402.06 mg. ingested. These results fail to show any significant difference in the amount of iron retained by the patient with hemochromatosis as compared to the control subjects.

CONCLUSIONS

Three cases of hemochromatosis in advanced stages of the disease retained no more iron than did two cases of diabetes mellitus. This was true not only when the iron was obtained from the food alone but also when an additional 340 mg. of iron was given in the form of iron and ammonium citrates. We present these findings as evidence that in the late stages of hemochromatosis there is no abnormal iron retention.

The results obtained in Case 4 are more difficult to interpret since the studies were of such short duration. For the single period of observation an unusually large iron retention occurred, distinctly greater than that in other cases of hemochromatosis or control subjects and also greater than we have obtained under similar conditions in other patients. It is obvious from the results of tissue analysis that retention of unusually large amounts of iron must occur at some time in the development of hemochromatosis. The clinical recognition of the disease is difficult or impossible in the early stages before evidence of pigmentation, cirrhosis of the liver, and diabetes make their appearance, so that iron balance studies will be difficult to obtain at that time. We were fortunate in obtaining one patient in a relatively early stage who had entered the hospital because of an entirely unrelated condition. He presented moderate pigmentation of the skin, slight enlargement of the liver and no glycosuria but a diminished glucose tolerance. The results from this one

patient cannot be considered as conclusive but suggest that iron is retained in excessive amounts in the early stage although this abnormal retention does not persist in the fully developed case.

SUMMARY

In advanced cases of hemochromatosis there was no evidence of abnormal iron retention.

In one case in an earlier stage of the disease, evidence of an unusually large iron retention was found.

REFERENCES

1. Rolleston, H., and McNee, J. W.: Cirrhosis with Haemochromatosis. Diseases of the Liver, Gall Bladder and Bile Ducts, ed. 3, London, 1929, The Macmillan Company, p. 332.
2. Muir, R., and Dunn, J. S.: The Iron Content of the Organs in Bronzed Diabetes (Haemochromatosis), J. Path. & Bact. **19**: 226, 1914.
3. Sheldon, J. H.: The Iron Content of the Tissues in Hemochromatosis With Special Reference to the Brain, Quart. J. Med. **21**: 123, 1927.
4. Mallory, F. B.: The Relation of Chronic Poisoning With Copper to Hemochromatosis, Am. J. Path. **1**: 117, 1925.
5. Mallory, F. B., Parker, F., and Nye, R. N.: Experimental Pigment Cirrhosis Due to Copper and Its Relation to Hemochromatosis, J. M. Research, **42**: 461, 1921.
6. Mallory, F. B.: Hemochromatosis and Chronic Poisoning With Copper, Arch. Int. Med. **37**: 336, 1926.
7. Althausen, T. L., and Kerr, W. J.: Hemochromatosis. II. A Report of Three Cases With Endocrine Disturbances and Notes on a Previously Reported Case. Discussion of Etiology, Endocrinology **17**: 621, 1933.
8. Labbe, M., and Petresco, M.: Alteration of Endocrine Glands in Diabetes Mellitus and Bronze Diabetes, Ann. d'anat. path. **11**: 761, 1934.
9. Howard, C. P., and Stevens, F. A.: The Iron Metabolism of Hemochromatosis, Arch. Int. Med. **20**: 896, 1917.
10. McClure, C. W.: Metabolism in a Case of Hemochromatosis, Arch. Int. Med. **22**: 610, 1918.
11. Garrod, A. E., Gaskell, J. F., Sladden, A. F., MacKenzie Wallis, R. L., and Varle, P. T.: A Contribution to the Study of Bronzed Diabetes, Quart. J. Med. **7**: 129, 1913.
12. Dry, T. J.: Hemochromatosis: Its Relation to the Metabolism of Iron and Copper, Minnesota Med. **17**: 301, 1934.
13. Fowler, W. M., and Barer, Adelaide P.: The Retention and Utilization of Orally Administered Iron, Arch. Int. Med. **59**: 561, 1937.
14. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorous, J. Biol. Chem. **66**: 375, 1925.
15. Reis, F., and Chakmakjian, H. H.: Colorimetric Method for Quantitative Determination of Iron in Blood in the Form of Dispersed Prussian Blue, J. Biol. Chem. **92**: 59, 1931.
16. Barer, Adelaide P., and Fowler, W. M.: The Influence of Gastric Acidity and the Degree of Anemia on Iron Retention, Arch. Int. Med. **59**: 785, 1937.

LABORATORY METHODS

THE WELTMANN SERUM COAGULATION REACTION*

Preliminary Report

S. A. Levinson, M.D., R. I. Klein,† M.D., and P. Rosenblum, M.D.,
Chicago, Ill.

THE altered colloidal stability of the blood serum which occurs in various pathologic conditions has been used as the basis of many serodiagnostic tests. Most of these tests have been discarded, because they either added little to the study of a case or were inconclusive in their interpretation. We might mention among others, the Daranyi reaction, the Matefy test, and Verne's resorcinol reaction. Several others, such as the formol-gel test of Gaté and Papacostas and Kürten which is used in the diagnosis of Leishmaniasis, leprosy, and subacute bacterial endocarditis, and the Takata-Ara reaction, which is regarded as an aid in detecting cirrhosis of the liver, are viewed with favor in some quarters.

In 1930, Weltmann[1] introduced the serocoagulation test which bears his name. Although the exact mechanism of the test is not established, it is based on the knowledge that the normally hydrophilic serum proteins in the disperse phase become hydrophobic as a result of heat coagulation, and if the pH and electrolytic concentration are proper, flocculation occurs.

Basing his investigations on the previous work of Rosenow, Pauli, Handowsky, and others, Weltmann found that when normal human serum is diluted 50 times with distilled water and heated in a boiling water-bath, no coagulation occurs and the contents of the tubes remain clear. When normal serum is diluted with tap water or physiological saline and heated, a cloudiness develops. When, however, serum from a patient with cirrhosis of the liver is diluted with physiological saline and heated, a flocculation occurs. If calcium chloride, barium chloride or magnesium chloride is added to diluted normal serum flocculation occurs when the mixture is heated in a boiling water bath. Weltmann found that when bivalent cations were used to bring about the proper electrolytic concentration much smaller amounts were sufficient than when univalent cations were used.

From these observations Weltmann evolved his test, the principle of which is to place constant quantities of serum with different concentrations of bivalent cations and then heat the mixtures in a boiling water bath.

*From the Departments of Pathology and Pediatrics, University of Illinois, College of Medicine and the Cook County Hospital.

Received for publication, January 26, 1937.

†Mead Johnson fellow.

The *technique* of the test is as follows: From a stock solution of 10 per cent $CaCl_2.6H_2O$, ten dilutions consisting of 0.1 per cent, 0.09 per cent, 0.08 per cent, 0.07 per cent, 0.06 per cent, 0.05 per cent, 0.04 per cent, 0.03 per cent, 0.02 per cent, and 0.01 per cent are made up. These dilutions are numbered from 1 to 10 beginning with the strongest concentration. Ten small test tubes, such as are used in the Wassermann reaction, are placed in a metal rack and numbered in order from 1 to 10. Into each tube is pipetted 5 c.c. of the similarly numbered $CaCl_2$ solution and 0.1 c.c. of unhemolyzed serum. The tubes are then shaken so that the contents are well mixed and placed in a boiling water bath for 15 min. They are then removed and the reaction can be immediately determined. The contents of the tubes may be clear, faintly opalescent or turbid; or there may be a flocculation. The last reaction is the only one noted. There is usually a sharp and easily noted difference between the flocculation and turbidity.

The number of tubes in which coagulation occurs is designated by Weltmann as the coagulation band (c.b. of that particular serum). If there is a very slight or doubtful flocculation in one tube the reaction is interpreted as being intermediate between that tube and the one before it. In normal serum the first six tubes usually show flocculation. Sometimes there is a slight or doubtful flocculation in the seventh tube. The normal coagulation band is therefore 6 to 6½ and is remarkably constant. This means that normal human serum diluted fifty times with calcium chloride solution and heated in a boiling water bath for fifteen minutes will coagulate or flocculate if the concentration of the calcium chloride is more than 0.04 per cent. If the coagulation band is less than six, Weltmann speaks of the reaction as showing a shift to the left, whereas, if the coagulation band is seven or greater the reaction indicates a shift to the right.

In pathological conditions the coagulation band may be lengthened or shortened. Inflammatory and exudative processes cause a shift to the left or shortening of the coagulation band, while fibrotic processes and those producing hepatic parenchymal damage lead to a shift to the right or lengthening of the coagulation band. Where two divergent processes such as exudation and fibrosis occur together, the coagulation band may be normal.

The Weltmann reaction has been studied by a number of European investigators all of whom have been favorably impressed by its possibilities, but it has attracted little attention in this country. Weltmann, himself and together with Sieder[2] found a shortened coagulation band in pneumonia, abscesses, rheumatism, and exudative tuberculosis; while a lengthened coagulation band was observed in cardiac decompensation, cirrhosis of the liver, and fibrotic tuberculosis. The reaction has been studied in syphilis by Sicher and Wiedmann,[3] in dermatology by Midana and Leone,[4] in gynecology by Purper[5] and by Ypsilanti and Kyriakis,[6] and in arthritis by Hennes and Kemen.[7] Several investigators have studied the Weltmann reaction in tuberculosis. Among these are Pongar,[8] Voigtländer,[9] Ekhart,[10] Friedmann,[11] Zambrano,[12] Makitra and Tyndel,[13] Nannini and Marani,[14] and Kisch,[15] Kraemer,[16] and Massobrio and de Michelis,[17] have investigated the test in diseases of the liver and

biliary tract. The reaction has also been determined in a variety of clinical conditions by Scherleitner,[18] Carriere, Martin and Dufossé,[19] Purper,[20] Kretz and Kudlac[21] and Teufl.[22]

Although up to the present time we have determined the Weltmann reaction in approximately twelve hundred children and adults and in a variety

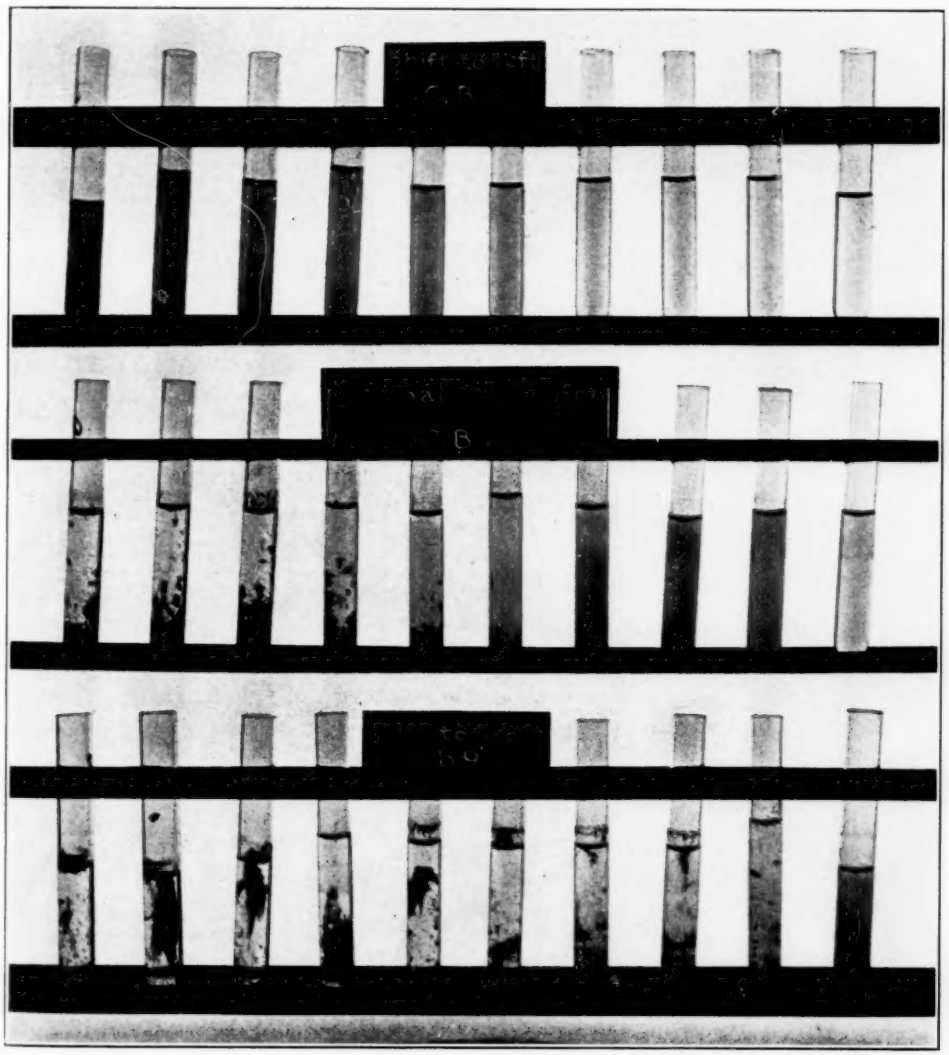


Fig. 1.—Middle row shows normal coagulation band (*C.B.*) and flocculation ends in Tube 6. Upper row shows no coagulation in any of the tubes; the coagulation band (*C.B.*) is zero. The lower row shows flocculation band (*C.B.*) 9.

of clinical conditions, our detailed observations will be presented at another time. Our purpose in this preliminary report is to present our results in a very general fashion, to call attention to the possibilities of this test as a laboratory aid in diagnosis and prognosis, and to briefly compare the Weltmann reaction with the sedimentation rate.

Our material consists of patients admitted to the Cook County Hospital and the Research and Educational Hospital during the past eighteen months. Cases in which a definite clinical diagnosis could not be made have been

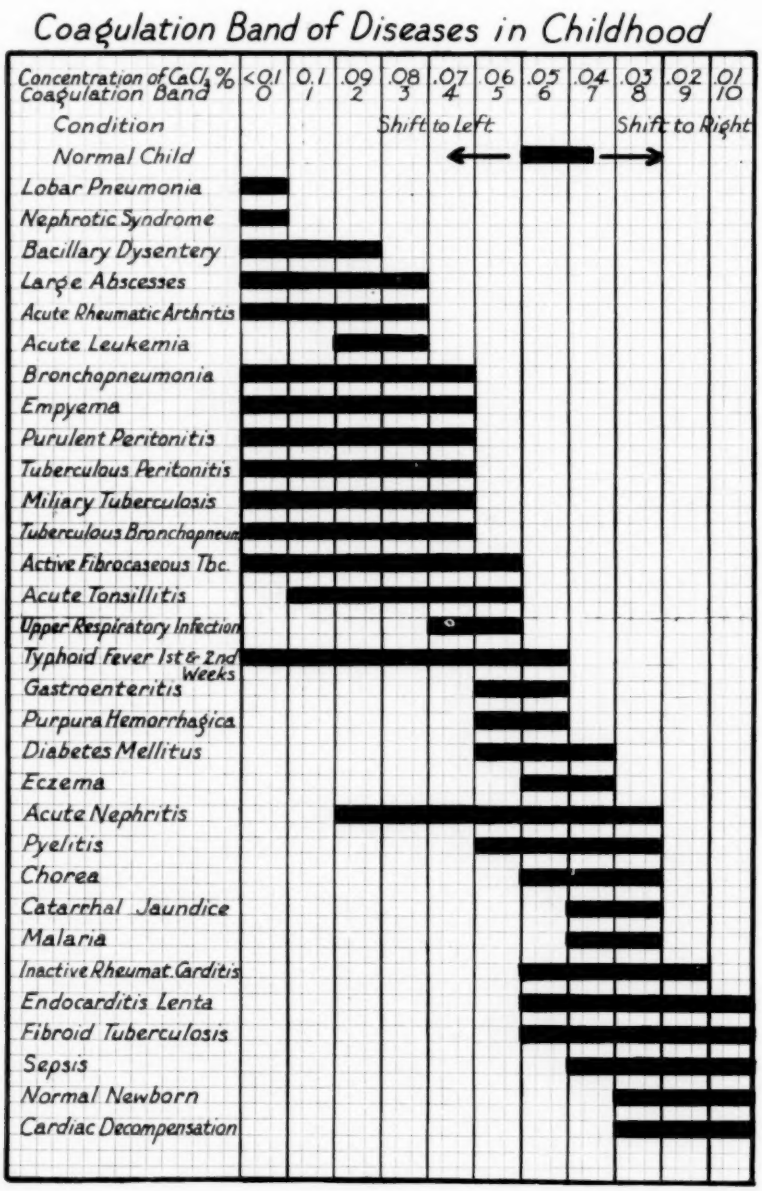


Fig. 2.

eliminated from the present series. We used Weltmann's original technique throughout, and the sedimentation rate was determined by the method of Wintrobe and Landsberg[23] so that corrections could be made for the red cell volume.

Although the maternal blood in uneventful pregnancies gives a normal coagulation band of six at the time of delivery, the cord blood of the newborn infant gives a lengthened coagulation band of seven to ten. This has been previously noted by Purper and may be cited as additional evidence of the functional weakness of the hepatic parenchyma in the newborn. In our series we could not correlate the extent of the shift to the right of the coagulation band with the degree of physiologic icterus.

In both children and adults the acute infections, as a rule, give shortened coagulation bands. This is more noticeable in children than in adults because in the latter physiological or subclinical fibrosis of various parenchymal tissues may tend to offset somewhat the degree of shortening of the coagulation band. Kretz and Kudlac have pointed out that the coagulation band tends to increase in the aged without the presence of demonstrable disease. Arteriosclerosis, myocardial fibrosis, and slight cirrhotic changes in the liver are enough to cause a noticeable shift to the right.

In the accompanying chart (Fig. 2) we have graphically illustrated the range in the coagulation band in some of the more frequent conditions encountered in childhood. In general, the acute inflammatory and exudative processes give a shift to the left, while chronic diseases give variable reactions depending upon the stage of the disease at the time of the examination. Septic and fibrotic conditions give a shift to the right.

In uncomplicated lobar pneumonia a coagulation band of zero (no coagulation with 0.1 per cent $CaCl_2$) was obtained in 107 out of 115 cases. After the onset of clinical resolution, the coagulation band as a rule returned slowly to normal, but did not reach a value of 6 for ten to fourteen days. In a few cases the coagulation band jumped from 0 to 5 in two or three days, but we are not prepared to state that there is any relation between the manner in which the Weltmann reaction returned to normal, and the type of resolution. Purulent complications caused the shift to the left to be prolonged, but uneventful otitis media had no effect on the coagulation band. In lobar pneumonia the Weltmann reaction cannot be used as a prognostic aid because there is no correlation between the coagulation band and the height of the fever, degree of toxicity, character of the etiologic organism, site of the lung involved, age of the patient, white blood count, or other elements determining the clinical outcome. The character of the pneumonic exudate is such that it reflects itself in the serum as a greatly reduced coagulation band.

In bronchopneumonia, empyema, and lung abscess, there is also a shift to the left, but usually not to the extent seen in lobar pneumonia. The shortening of the coagulation band in these conditions is usually proportional to the extent of the suppurative process, and the coagulation band varies from 0 to 4. Whereas in bronchopneumonia and lobar pneumonia the coagulation band usually does not go beyond normal during healing, in empyema and at times in lung abscess, there is seen a shift to the right and the coagulation band goes to Tube 8 or 9. This is in keeping with our knowledge of the anatomic changes taking place. There is considerably less fibrosis in the heal-

ing of a bronchopneumonia than there is in an empyema. In bronchiectasis the coagulation band varies and depends upon the predominating process at the time. If there is much suppuration and the condition is practically an abscess, there is a shortened coagulation band, but in the chronic types where fibrosis plays a predominant rôle, the coagulation band is lengthened.

In the upper respiratory infections including acute bronchitis, the coagulation band is either normal or only slightly shortened, but in tonsillitis, streptococcus sore throat and diphtheria, there is usually a distinct shift to the left.

In acute rheumatic arthritis and carditis, the coagulation band is shortened usually in proportion to the clinical severity of the condition. In chorea, uncomplicated by other rheumatic manifestations, the coagulation band is usually normal or may even be slightly increased. Although in both acute rheumatic arthritis and carditis the coagulation band goes back toward normal when the clinical signs of activity begin to lessen, in the case of an uncomplicated arthritis the coagulation band does not go beyond Tube 6 or 7, while in the cardiac cases it goes to Tube 8 or 9. This again fits in with our clinical knowledge that there are no fibrotic changes in rheumatic polyarthritis, while in rheumatic carditis fibrotic changes in the endocardium and pericardium constitute the normal sequelae to the acute inflammatory reaction.

In sepsis and in subacute bacterial endocarditis the coagulation band is in Tubes 6 to 9, and a similar shift to the right is also found in cardiac decompensation, malaria, and catarrhal jaundice.

The Weltmann reaction reflects the anatomic-pathologic changes in tuberculosis, if the condition shows itself either as an exudative or productive process. Although both of these processes may exist in the same patient at the same time, the coagulation band is a distinct aid in denoting the predominant trend of the condition, and is an important guide in the prognosis. A moderately shortened coagulation band is obtained in the active period of the primary complex, and a greatly shortened coagulation band in a rapidly progressive case of tuberculous pneumonia, and tuberculous meningitis. In tuberculous adenitis the coagulation band is slightly diminished, while in bone and joint tuberculosis it is usually normal or slightly increased. When fibrosis supersedes exudation in any type of tuberculosis the coagulation band becomes increased.

Comparison With the Sedimentation Test.—We have simultaneously determined the sedimentation rate by the method of Wintrobe and Landsberg with the Weltmann reaction in a variety of conditions, and also repeated the two tests several times in the same patient. In general, the acute inflammatory conditions and the exudative stages of the chronic infections like tuberculosis and rheumatic fever give both shortened coagulation bands and rapid sedimentation rates. However, there is no parallelism between the two tests. In Table I we present the findings in fifty unselected cases. We note that in some of the conditions like carcinoma which may give a distinct shift to the left in the Weltmann test, the sedimentation rate may be normal; while in

TABLE I

COMPARISON OF WELTMANN'S COAGULATION BAND WITH THE SEDIMENTATION TEST IN 50 UNSELECTED CASES

DIAGNOSIS	SED. MM. 1 HR.*	C. V.	CORR. SED.	WELT.
Acute pyelonephritis	60	35	35	0
Ca. of cervix with metastases	29	27	2	0
Perforating peptic ulcer	2	52	7	0
Unresolved pneumonia	61	36	40	0
Cellulitis of left arm	2	38	2	0
Perforating duodenal ulcer P. O.	23	51	28	0
Perforated gastric ulcer P. O.	44	48	45	0
Strangulated hernia and Perit. P. O.	47	44	44	1
Arteriosclerotic kidney, impending uremia	49	47	49	1½
Thrombophlebitis and scurvy	43	48	44	2½
Diabetic gangrene	65	30	29	2½
G. C. arthritis	45	44	40	3
Ulcer and apical tuberculosis	53	42	43	3
Preuremia and chronic nephritis	72	26	27	3
Acute lichen planus	45	56	61	3
Acute rheumatic arthritis	31	46	32	3½
Hypertension and diabetes	13	52	19	4
Lymphangitis	28	43	23	4
G. C. arthritis and toxic derm.	46	44	40	4
Blastomycosis	29	41	21	4
Ca. of testes with metastases	48	48	50	4
Chronic ulcerative colitis	45	38	29	5
Hypertrophic arthritis, diabetes	34	43	28	5
Typhoid fever, 6th week	42	41	32	5
Duodenal ulcer	36	46	34	5
Lymphogranuloma inguinale	57	38	39	5
Hydronephrosis	50	45	55	5
Syphilis I and II	41	49	45	5
Granuloma ing. and syphilis III	53	34	24	5
Carcinoma of rectum	54	26	18	5½
Carcinoma of colon	61	24	18	5½
Uremia	47	44	40	5½
Tertiary syphilis	29	44	25	6
Chronic nephritis	33	36	17	6
Carcinoma of stomach	54	40	41	6
Duodenal ulcer	31	37	16	6
Hyperthyroidism	13	45	11	6
Perf. duodenal ulcer with sub. abscess	58	30	25	6
Dermatitis herpetiformis	26	55	38	6
Tertiary syphilis	22	50	26	6
Tertiary syphilis	28	44	24	6
Old cong. syphilis	5	47	5	6
Tertiary syphilis	4	49	4	6
Chronic eczema	56	50	63	6½
Tertiary syphilis	0	52	0	7
Yeast dermatitis	22	54	32	7
Catarrhal cholangitis	43	40	31	9
Lymphogranuloma inguinale	45	55	68	9
Tertiary syphilis	41	42	33	9
Carcinoma of esophagus	51	41	39	10

*Code: Sed. mm. 1 hr., Sedimentation millimeters in one hour; C. V., cell volume; Corr. Sed., corrected sedimentation time; Welt., Weltmann.

other conditions like lymphogranuloma inguinale in the fibrotic stage which gives a shift to the right in the coagulation band the sedimentation rate is very rapid.

In tuberculosis, the Weltmann test may be of greater value than the sedimentation rate, and we illustrate this observation in Tables II, III, and IV. In Table II the findings obtained in a twelve-year-old colored girl with ulcera-

TABLE II

FIBROULCERATIVE TUBERCULOSIS (RAPID IMPROVEMENT)

DATE	SED. MM. 1 HR.	C. V.	CORR. SED.	WELT.
7/21	60	34	46	5
7/28	65	29	39	4
8/ 6	67	27	34	4
8/13	67	27	34	4
8/20	62	31	41	7
8/27	56	38	52	7
9/ 3	38	40	38	8
9/10	52	37	46	9
9/24	49	36	41	9
10/ 1	47	36	39	8
10/13	47	38	44	9
10/22	48	39	46	10
10/29	40	39	38	10
11/ 6	50	37	43	9
11/12	41	38	37	9
11/19	44	38	40	9

tive pulmonary tuberculosis are recorded. At the time of admission the girl had a fever, the Weltmann reaction gave a coagulation band of 4 and the sedimentation rate was very rapid. This girl made a very good improvement and clinically was well enough to be discharged under supervision in four months. Her sedimentation rate improved very slowly but the coagulation band went fairly rapidly to the right indicating prompt and predominating fibrosis which was in keeping with her clinical course. If one were to be guided on the sedimentation rate alone, her improvement would not seem to be as rapid as both her clinical condition and her Weltmann reaction indicated.

TABLE III

FIBROULCERATIVE TUBERCULOSIS (VERY SLOW IMPROVEMENT)

DATE	SED. MM. 1 HR.	C. V.	CORR. SED.	WELT.
7/21	54	39	52	4
7/28	51	40	51	4
8/ 6	52	37	45	4
8/13	55	37	48	4
8/20	54	38	50	4½
8/27	53	36	44	3½
9/ 3	34	40	34	4
9/10	55	37	49	4
9/24	49	37	43	4
10/13	52	36	44	5
10/22	54	36	45	4
10/29	54	36	45	5
11/ 6	54	35	43	5
11/12	53	36	44	5
11/19	53	35	42	5
12/ 2	53	34	40	6

Table III illustrates the findings in á thirteen-year-old Mexican girl with predominating unilateral tuberculosis. Her clinical condition for three months was practically stationary, and her Weltmann reaction persistently gave a coagulation band of 4, while the sedimentation rate was always rapid. Pneumothorax was instituted on the affected side with gratifying clinical results, and her coagulation band began to move to the right. The sedimentation rate, however, continued to be rapid for some time.

In Table IV we have the results in a seven-year-old Mexican girl with a rapidly progressive caseous tuberculosis of endocanalicular distribution from the breaking down of the primary lesion. The coagulation band remained low during the whole course of observation, and before death it became zero. The sedimentation rate fluctuated from one level to another and at times might have been interpreted as indicating improvement.

TABLE IV

BRONCHOGENIC DISSEMINATION OF A TUBERCULOUS PRIMARY LESION (DEATH)

DATE	SED. MM. 1 HR.	C. V.	CORR. SED.	WELT.
7/28	67	28	38	4
8/ 6	30	26	8	2
8/13	60	25	28	3
8/20	62	27	33	4½
8/27	46	29	24	2
9/ 3	39	29	19	3
9/10	57	29	33	2½
9/24	38	30	20	0

TABLE V

ACUTE RHEUMATIC CARDITIS

DATE	SED. MM. 1 HR.	C. V.	CORR. SED.	WELT.
8/27	63	29	38	2
9/ 1	48	27	24	2½
9/ 8	66	30	46	5
9/10	61	34	53	5
9/15	55	39	53	6
9/24	52	37	46	6
10/ 1	56	35	46	2
10/13	30	46	40	7½
10/22	16	44	22	8
10/29	11	45	17	9
11/ 6	28	38	25	6
11/12	15	41	16	6

It is well known that in cachectic or terminal conditions the sedimentation rate may approach normal. The Weltmann reaction in our experience indicates more reliably the condition of the patient, and if the process is an exudative one, there is always a marked shift to the left. We cannot overemphasize the fact that if exudative and fibrotic processes coexist, they may interfere with each other in the outcome of the Weltmann reaction. In such cases the sedimentation rate may give additional aid in conjunction with the clinical picture.

The sedimentation rate has been extensively used as an index of activity in rheumatic fever and those investigators who have adopted it routinely feel that it is the single best laboratory observation at hand. The detailed findings in our studies on the Weltmann reaction in rheumatic fever will be presented elsewhere but we may briefly give our results here. In acute rheumatic carditis the rapid sedimentation rate is accompanied by a shortened coagulation band. The sedimentation rate returns very slowly to normal but the coagulation band increases quite rapidly, and frequently moves to the right. When the patient is afebrile, has a normal pulse rate, and physical findings

of activity have disappeared, the sedimentation rate may still be rapid and the Weltmann reaction normal or to the right. Although for practical purposes, the sedimentation rate may be a better guide as to the extent of freedom the patient may be allowed, we feel that the coagulation band indicates more correctly the pathologic-anatomic picture. We believe that when the rheumatic patient shows an increased coagulation band and a rapid sedimentation rate without other signs of active infection, fibrosis is present, and that he is convalescing, but is not yet completely recovered. A good number of patients with rheumatic carditis have recurrent exacerbations of the infection before the first attack has subsided. In such cases the sedimentation rate is still rapid from the first attack when the fresh infection occurs, and thus does not indicate what is taking place, although the temperature and physical findings indicate renewed activity. In the above examples the Weltmann reaction is a distinct aid because it had previously gone toward the right and now with the fresh exudative process it returns to the left. Table V illustrates this very well. A seven-year-old girl entered the hospital with recurrent acute rheumatic carditis. The coagulation band was 2 and the sedimentation rate very rapid. Her temperature soon declined, her pulse rate slowed, she improved clinically and her coagulation band became 6. She then developed a new exudative process, and her temperature went up to 103° F., her pulse rate 130, and she complained of precordial pain. At this time her coagulation band went back to 2, but her sedimentation rate showed little change since it was still very rapid.

In subacute bacterial endocarditis the sedimentation rate is the same as it is in acute rheumatic carditis, but the Weltmann reaction is normal, or shows a shift to the right instead of a shift to the left. There are no foci of exudation in sepsis lenta as compared to rheumatic carditis, and the liver damage frequently coexisting pulls the coagulation band to the right. In cases in which a clinical diagnosis is not definite, the determination of the coagulation band may be a great help.

Very little is known concerning the mechanism of the Weltmann reaction. Nannini and Marani believed that an increase in globulin and a decrease in albumin is necessary for a shortened coagulation band. Carriére, Martin and Dufossé, however, could find no relationship between the total protein and albumin-globulin ratio. We have determined the total protein and albumin-globulin ratio in forty cases and our findings agree with those of Carriere and others. Where the total protein is high, and the serum globulin shows a marked increase, the coagulation band may be lengthened. In nephrosis and in some cases of cirrhosis of the liver the quantitative estimations of the serum proteins give identical results. Both show a low total serum protein with a low albumin and a reversal of the albumin-globulin ratio, yet nephrosis gives characteristically a very low coagulation band while cirrhosis of the liver gives a high coagulation band. Kretz and Kudlac found the chloride and calcium contents of the serum to have no effect and attributed the reaction to a qualitative change in the serum proteins. This is substantiated by our own findings. In a case of tetany, for instance, with a serum calcium of 6.2 mg. per 100 c.c. of blood, the coagulation band showed a slight shift to

the right instead of a shift to the left. That the pH has little influence is seen when we find normal coagulation bands in diabetic acidosis. Weltmann found that the addition of bile does not alter the reaction, and in cases of obstructive jaundice with slight cirrhotic changes in the liver the coagulation band may be normal. Hemolysis definitely lengthens the coagulation band and it is essential that the serum be unhemolyzed. Fever in patients has no relation to the Weltmann reaction and in several cases of chorea that received inductotherm therapy the induced hyperpyrexia did not change the coagulation band. Fibrinogen has no influence on the length of the coagulation band since serum and not plasma is used in the test. So far, in preliminary experiments, we have not been able to demonstrate any difference between the serum proteins of blood giving a coagulation band of zero and those giving a coagulation band of 9 or 10 when the serum is used as an antigen, and precipitin reactions are made.

SUMMARY

The Weltmann reaction is not a specific diagnostic test for any disease, but detects exudative and fibrotic processes, and parenchymal damage to the liver. When properly interpreted it may be used as an aid in differential diagnosis when the clinical findings are not clear. We have found it to be of value in the diagnosis of sepsis lenta, in ruling out pneumonia when an x-ray was not available, and in denoting the presence of suppuration when it was not clinically discernible at the time. When a patient has a definite febrile disease and the diagnosis cannot be made, a normal or lengthened coagulation band helps to rule out pneumonia, acute rheumatic fever, active tuberculosis, accumulations of pus, and other exudative processes. A febrile reaction with a normal or lengthened coagulation band leads one to think of sepsis, malaria, and sometimes typhoid fever. When the Weltmann reaction is determined at frequent intervals it is a definite aid in assisting the clinician in the course of the disease, when the underlying condition is one characterized pathologically by exudation, with subsequent resolution or fibrosis.

REFERENCES

1. Weltmann, O.: Über die Spiegelung exudatibetzündlicher und fibroser Vorgang in Blutserum, Med. Klin. **26**: 240, 1930; Zur Leberpathologie, Wien. Klin. Wchnschr. **43**: 1301, 1930.
2. Weltmann, O., and Sieder, B.: Die Bedeutung des Koagulationsbandes (K. B.) für der Diagnose den Leberkrankheiten, Wien. Arch. f. Inn. Med. **24**: 321, 1934.
3. Sicher, G., and Wiedmann, A.: Die Koagulationsband von Weltmann bei Syphilis, Med. Klin. **30**: 1664, 1934.
4. Midana, A., and Leone, R.: La reazione di Weltmann in dermatologia, Dermosiflografo **9**: 494, 1934.
5. Purper, F. G.: Die Weltmannsche Koagulationsband bei Adnexentzündungen, Monatschr. f. Geburtsh. u. Gynäk. **97**: 138, 1934.
6. Ypsilanti, H., and Kyriakis, L.: Über unsere Erfahrungen mit dem Weltmannschen Koagulationsband in der Gynäkologie, Zentralbl. f. Gynäk. **58**: 1122, 1934.
7. Hennes, H., and Kemen, A.: Der Wert der Weltmannschen Reaktion in der Rheumatologie, Klin. Wchnschr. **15**: 378, 1936.
8. Pongar, F.: Über die Weltmannsche Reaktion, Ztschr. f. Tuberk. **73**: 193, 1935.
9. Voigtländer, W.: Untersuchungen über die Serumkoagulationsreaktion von Weltmann bei Lungentuberkulose, Klin. Wchnschr. **13**: 1718, 1934.
10. Ekhart, W.: Die Weltmannsche Serumkoagulation bei Tuberkulose, Med. Klin. **27**: 772, 1931.

11. Friedmann, E.: Beiträge zur Koagulationsreaktion nach Weltmann bei der Tuberkulose, Klin. Wchnschr. **14**: 132, 1935.
12. Zambrano, E.: Sul valore della siero-coagulazione di Weltmann nei bambini tubercolotici, La Pediatria **40**: 1279, 1932.
13. Makitra, A., and Tyndel, M.: Über den Wert der Weltmannschen Reaktion bei der Beurteilung der Lungentuberkulose, Beitr. z. Klin. d. Tuberk. **84**: 265, 1934.
14. Nannini, G., and Marani, B. D.: La siero-coagulazione di Weltmann nella tuberculosi polmonare nei delle emazie, Riv. di pat. e clin. d. tubere. **6**: 515, 1932.
15. Kisch, F.: Über die Elektrolytschwelle der Blutserumeiweiss-Koagulation Kreislaufkranken, Klin. Wchnschr. **10**: 1172, 1931.
16. Kraemer, M.: The Weltmann Test in Diseases of the Liver, Am. J. Dig. Dis. Nutr. **2**: 14, 1935.
17. Massobrio, E., and de Michelis, U.: La Sero-coagulation de Weltmann dans les Maladies du foie par roppart à l'equilibre proteique du serum, Minerva Med. **1**: 147, 1934; Presse méd. (Rev. des Journ.) **42**: 190, 1934.
18. Scherleitner, P.: Beiträge zur Serum Koagulation nach Weltmann, Wien. Med. Wchnschr. **82**: 1276, 1932.
19. Carriere, G., Martin, P., and Dufossé, M.: La Sero-Coagulation de Weltmann en Clinique, Presse méd. **43**: 1649, 1935.
20. Purper, G.: Die Klinische Bedeutung des Weltmannschen Koagulations-bandes, München. med. Wchnschr. **82**: 1124, 1935.
21. Kretz, J., and Kudlac, L.: Untersuchungen über das Verhalten der Serumeiweisskörper mittels der Weltmannschen Reaction, Ztschr. f. Klin. Med. **127**: 590, 1935.
22. Teufl, R.: Der Diagnostische Wert der Serumkoagulation nach Weltmann, Wien. Arch. f. Inn. Med. **28**: 305, 415, 1936; **29**: 37, 297, 1936.
23. Wintrobe, M. M., and Landsberg, J. W.: A Standardized Technique for the Blood Sedimentation Test, Am. J. M. Sc. **189**: 102, 1935.

MUSEUM MOUNTINGS*

ERIC A. FENNEL, M.D., HONOLULU, T. H.

WE HAVE, for some time, been using in The Clinic (Honolulu), a method for the mounting of museum specimens, particularly in surgical pathology, that has proved to be eminently satisfactory. It is especially well adapted to show the plane surfaces of sliced specimens. The method consists simply of making a box for the specimen out of two sheets of glass, for top and bottom and narrow strips of glass for the sides, the whole cemented together by asphalt. It is in some respects similar to the methods advocated by Davis and Amlosh[1] and Aronson and Taub,[2] but differs from their methods in so many essential details that it is considered worthy of recording.

It has many advantages and only a few disadvantages. It is very cheap; even the quite large specimens may be mounted with less than fifteen cents' worth of material. No unusual dexterity is required for making the boxes, large or small. They should be as permanent as an Egyptian mummy, for the glass and asphalt are ageless. The finished specimen has about the shape and weight of a thin book, uses up a minimum of space and may be filed on a book shelf, one specimen thus protecting the other from the deteriorating influence of light. Both surfaces of the specimen are visible. To us it seems an almost ideal method.

These boxes are, however, not useful in mounting large specimens in which the exhibition of contour is essential, as in a heart, for example. Smaller specimens, tubes, ovary, appendix and similar ones, less than two inches in diameter, may be advantageously housed in modifications of these boxes which we routinely use.

This method was, in our hands, the outgrowth of one formerly employed, which in itself was an outgrowth of the old-fashioned watch crystal method, both of which have been abandoned by us. In the former method the properly fixed and trimmed specimen was mounted in one-half of a Petri dish, embedded in a thin layer of formalinized gelatin. This was then backed by paraffin and an additional layer of plaster-of-Paris for the sake of stability. Such mountings were inconvenient to handle, set on edge they tended to roll, they very frequently developed leaks and had to be remounted because the gelatin dried out, the formalin in the gelatin slowly dissipated the natural colors and the gelatin itself, on aging, took on a brown color.

Directions for mounting, by our method, a specimen, say a slice of liver to show carcinomatous metastases, follow:

Cut slabs of liver not more than an inch thick, wash and drop into Solution No. 1 of Robertson and Lundquist,[3] though any standard solutions, such as Kaiserling or Pick's may be uesd. This solution consists of:

Potassium acetate	85 gm.
Potassium nitrate	45 gm.
Chloral hydrate	80 gm.
Formaldehyde	444 c.c.
Water	4000 c.c.

*From the Department of Clinical Pathology, The Clinic.
Received for publication, January 26, 1937.

For this purpose use a jar with an inlet and outlet, the inlet tube going to the bottom of the solution in the jar. On the outlet attach a small-bore hypo needle. Attach the inlet to the illuminating gas cock and bubble carbon monoxide through the solution for an hour or two, lighting the gas at the hypo needle outlet to avoid gas poisoning in the laboratory. An 18 gauge needle will properly regulate the rate of the bubbles, though a screw pincheock may be substituted. The monoxide preserves well the blood red color.

Leave the specimen in the gas saturated solution for twenty-four to forty-eight hours, then wash in running water for four to six hours. The specimen is now trimmed to size—say 4 by 5 inches or 5 by 7. It is then sliced thin by hand or on the macrotome, a board with two glass rods of about one-quarter inch or less in diameter, mounted at a convenient distance from each other, which serve to guide a long, thin, very sharp knife. Such knives may be bought in a cutlery for fifty cents. These thin slabs are then transferred to the Solution No. 2, which consists of:

Potassium acetate	400 gm.
Chloral hydrate	200 gm.
Glycerin	400 c.c.
Water	3600 c.c.

After twenty-four to forty-eight hours they are placed in the second change of this solution and left there twenty-four hours or longer until it is convenient to mount them.

Now prepare a suitable box by selecting 2 pieces of glass of proper size, say 5 by 7. From a large sheet of glass, such as is used for picture frames, cut long strips of glass of equal width, about one-fourth inch or slightly wider. Glass cutting looks so difficult and is so easy; a cheap wheel glass-cutter, a straight edge ruler and an assistant to help steady the ruler are all that is needed. After scratching the line, grasp the glass firmly each side of the end of the scratch at the edge and bend the glass away from the scratch; this yields a smooth fracture line.

Cutting these glass strips gets to be a fascinating amusement after a little practice. If you are afraid of a sheet of glass your glazier will cut it for you for a trifle. Every laboratory and hospital has a picture of Rokitansky, Virchow, some dead and forgotten founder or a useless framed diploma that could well yield up its glass to a more utilitarian purpose.

This glass strip is then cut into lengths, 2 pieces 4½ inches, 2 pieces 6½ inches. The diamond point pencil is more useful for this step than the wheel cutter. These pieces make the sides of the box. One of these pieces then has one corner ground down on an emery wheel or rough stone so that a filling needle may be inserted into the completed box. A rectangle is then made of these four strips of glass by sticking the ends together with bits of label paper. This rectangle is then set on one of the sheets of glass, centered and covered with the other sheet of glass. The pieces of the glass box are then held in position by applying 2 or 4 heavy paper clamps. We use ACCO Clamps No. 225 or 325; their cost is negligible. If the specimen is sliced very thin, a side wall for the box may be made by bending a piece of glass rod of suitable diameter into a rectangle with one incomplete corner.

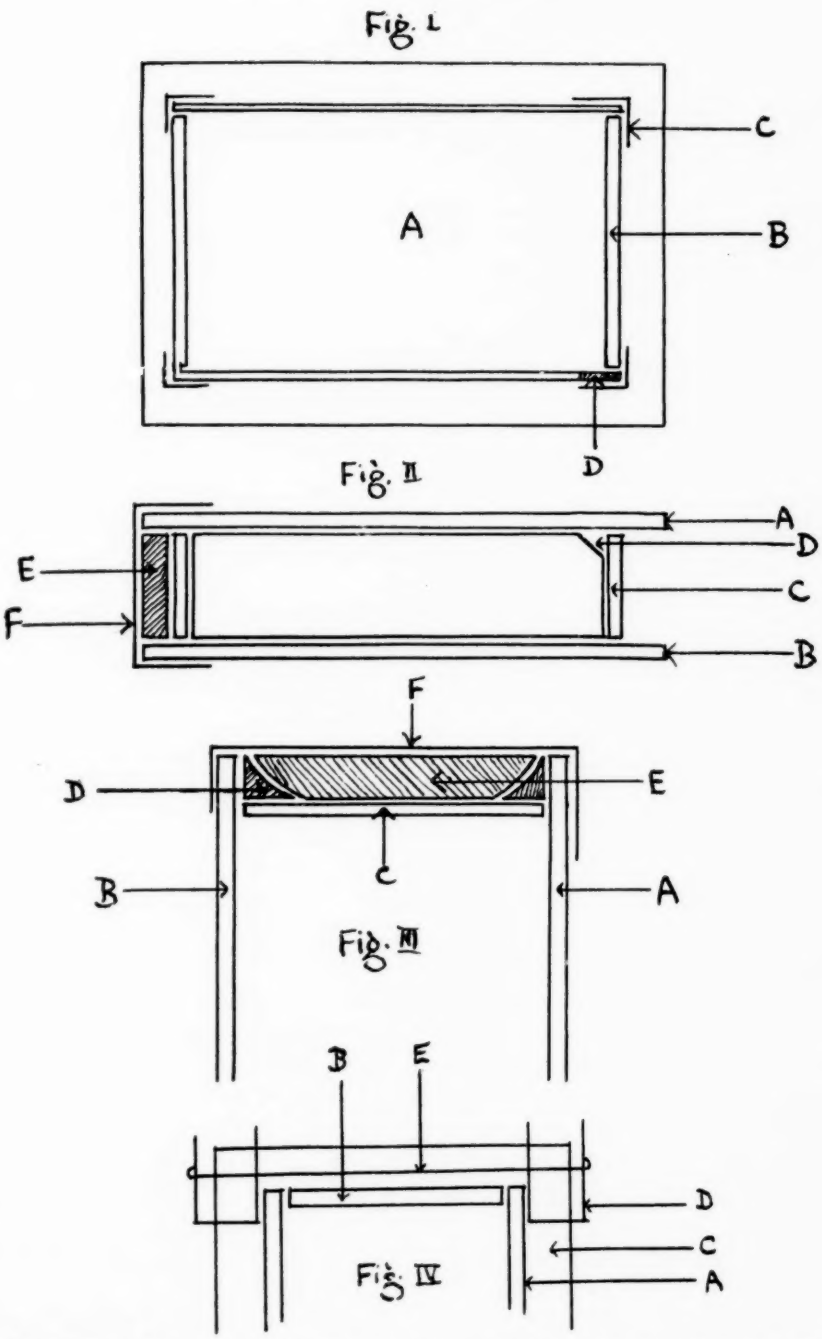


Fig. I.—Top view of box. *A*, Top and bottom sheets of glass superimposed. *B*, Side walls; strips of glass on edge. *C*, Gummed label paper. *D*, Ground off corner of one side wall.

Fig. II.—Side view of box. *A*, Top. *B*, Bottom. *C*, Side wall. *D*, Aperture created by missing corner. *E*, Asphalt. *F*, Passe-partout.

Fig. III.—Side view of deep box. *A*, Top. *B*, Bottom. *C*, Side wall. *D*, Asphalt. *E*, Plaster-of-Paris. *F*, Passe-partout.

Fig. IV.—Box of edge, view from the top side, showing bent tin held in place by rubber band for pouring asphalt. *A*, Side wall. *B*, Side wall, bottom of trough to be filled. *C*, Top sheet of glass. *D*, Tin bent into U-shape. *E*, Rubber band.

The rectangle of side walls is then fixed to the bottom glass sheet by pouring a drop of molten asphalt into the groove made by the bottom and each of the sides. This step is, however, like parsley on fish; it may be omitted after a little practice; we use it only when preparing a number of boxes in advance. Firmer attachment is secured if the glass is previously temperately flamed and the poured drop, in turn, flamed. For this purpose we have fixed a hypodermic needle in the end of the gas hose which gives a convenient small brush of flame.

The clamps and lid are then removed. The specimen is mopped dry and trimmed to snugly fit the box. Should it be desired to show the cut edge of the capsule or if the specimen is too thin or too limp to stand alone, it is sewed to a piece of clear celluloid and the celluloid cut to snugly fit the box.

It is better to use the celluloid mount and a deeper box rather than a box whose depth is just that of the thickness of the slab of tissue; clamping the specimen between bottom and top immobilizes it but leaves unsightly air bubbles between glass and specimen.

The specimen having been inserted in the box, the lid is replaced and adjusted and the clamps are reapplied. The box is then set on edge, small pieces of bent tin are held in position at the ends of the troughs made by the side walls and the projecting top and bottom, by rubber bands, to prevent overflow spilling, the trough is gently flamed to rid it of condensation vapor, and it is then poured full of molten asphalt. The flame brush is gently applied so that the surface is level and so that the asphalt adheres closely to the glass. A strip of paper is then laid on the asphalt, pressed down with a flat surface and as soon as the asphalt is slightly cool the position of the box is reversed. In this manner all four edge troughs are poured with asphalt. A small piece of tin is so adjusted that the asphalt does not overflow the ground off corner of the one side wall, or this triangular hole is covered with a strip of gummed paper, taking care to mark the corner that has been so treated. With the clamps still in place the asphalt is allowed to cool and thoroughly set.

Ordinary domestic asphalt, such as is used in making and repairing highway pavement is preferred by us. It has a low melting point and good adhesion and is easy to handle, but has a tendency to flow after setting, that must be controlled. Genasco Asphalt (Refined Trinidad Lake Asphalt, Genasco Brand, The Barber Asphalt Co., Phila., Pa.) has a higher melting point, is more difficult to melt and handle and is more easily charred, but lacks this tendency to flow.

With the clamps still in place the box is now filled with Solution 2 through the aperture left by the ground-off corner of the one side wall. If it was covered by gummed paper, a hot needle suffices to penetrate the asphalt and puncture the paper. A gravity bottle with a large bore hypo needle and a pinch cock is ideal for the purpose and it is well to set the box up on edge in a photographic plate drying rack during filling. It is filled to overflowing, shaking the box to rid it of bubbles. The glass and asphalt about the aperture are dried, gently flamed with the flame brush and molten asphalt is poured from a spoon to complete the seal. If the "paper hot-needle" method was used it is best to roll a tiny stopper of asphalt between the fingers, insert it into the dried hole and assure sealing by use of a hot spatula.

With the clamps still in place, to prevent flowing of the asphalt in warm weather, the specimen is allowed to stand in various positions for several hours, testing for leaks. If none is found, the clamps are removed and the specimen is bound around the edge with a strip of passe-partout or, if the box is too thick, with two strips, like a lantern slide, the label and number are glued to the edge and the book-like mount is ready for the shelf to await exhibition.

We have subjected some of these specimens to rapidly alternating temperatures in the incubator and the ice box, thus simulating the summer temperatures of New York and the winter temperatures of Chicago and the specimens remained unaffected.

Asphalt yields no color on prolonged exposure to Solution 2 and since nothing but glass and asphalt are in contact with the specimen and the fluid, the mount should be permanent. What happens to the celluloid after many years remains to be seen.

When mounting a larger, contoured specimen, say a tubal pregnancy with ovary, of a thickness of an inch or more, a modification of this technique is employed. From the large sheet of glass a strip of 1½ or 2 inches in width is cut and then cut into suitable length for the side walls, one corner ground off, stuck together by label paper to make the rectangle which is then centered on the bottom sheet of glass. The celluloid mounted specimen is inserted, the lid placed in position and the clamps applied. The sides are then firmly fixed to the bottom only by pouring asphalt into the right angle trough made by bottom and side, flamed into adherence with the flame brush after which the same is repeated for the sides and top, being careful not to cover the ground off corner. With a spatula molten asphalt is generously daubed on the corners at the junction of the side walls covering the label paper. The box is then filled with Solution 2 in the usual fashion, sealed, and with the clamps still in place tested for leaks. The clamps are then removed and the mount placed in a paraffined cardboard box with hinged sides, made to fit the mount snugly and plaster-of-Paris is then poured around the four edges. Slow setting plaster may be daubed and smeared into this edge trough with a spatula, instead of casting. Its purpose is to prevent the flow of the asphalt in warm weather. When the plaster has set, any excess is cut away and trimmed, the clamps are re-applied and the plaster is allowed to dry for twelve to twenty-four hours. The specimen is then bound with black passe-partout. Such broader sided boxes stand up on edge securely without support, for exhibition purposes.

When we began this method we used metal side walls bent into proper shape and soldered at the joint. These metal side walls were then attached to the bottom glass with asphalt and their inner surfaces and edges were painted with a variety of substances. The best results were obtained by using a saturated solution of Genasco asphalt in xylol, but if one tiny spot was overlooked, the corrosive preserving fluid ate the metal to the production of a brown-black color in the fluid. Besides, painting is a nuisance and time consuming, and we have therefore abandoned the metal sides.

There follow some practical hints that may be useful:

In cutting side wall strips, choose rather thin glass and particularly a sheet of even thickness.

The wheel type of glass cutter, with a steady, firm pressure works like a charm, particularly if you dip it into turpentine before use. That, however, may be only a superstition.

In gluing together the side walls with cut labels, four headless wire nails, driven into a board : : thus, to hold up one side, simplifies the work. The corners should overlap, not just meet at the edge.

In melting the asphalt, stir constantly lest it char; melted it may be kept molten on an asbestos plate over a bunsen burner.

We use small tin cans, bent to have a pouring snout and nailed to a wooden handle for melting and pouring pots. Small quantities melt more readily than large amounts.

The hypo needle gas flame, the "flame brush," is invaluable; do not try to get along without it; it works like a soldering iron.

Old x-ray films are an excellent source of celluloid; the old, pre-prohibition ones are colorless and yield their emulsion readily to hot water, a little lye and elbow grease; the new ones have a blue-tinged emulsion that sticks closer than a brother.

In sewing the tissue to the celluloid, use a fully curved needle and white thread, put the needled stitches in the tissue, superimpose the celluloid, mark the stitch outlets, remove the celluloid and punch holes in the marked places. Pass the threads through the holes and tie. Use long ties; thread is cheap.

We use 4 standard sizes of glass sheets, 3¼ by 4 (lantern slide cover glasses at 30 cents a dozen), 4 by 5 and 5 by 7 old photographic plates, and 8 by 12 standard window pane glass at 12 cents each. These may be cut into odd sizes as in mounting strips of intestine. For very small specimens we use standard 3 by 2 microslides.

If a specimen has inadvertently been fixed in 10 per cent formalin, a great deal of the natural color may be restored by first thoroughly washing in running water, transferring then to 50 per cent alcohol for six to eight hours and then to 2 or 3 changes of Robertson and Lundquist's Solution 2.

Arrows pointing to the essential pathology enhance the appearance of the completed specimen; since it is fixed in position within the box, they may be put on the outer surface of the glass. White paint or enamel and a printing pen (Esterbrook makes such) make this step easy and neat. The specimen may be labeled and numbered in this way, since it is permanent, in addition to the typewritten label on the edge, which is more easily read on the shelf but is less permanent. More recently we have used a quick drying paint, to put the number and label directly on the celluloid mounting within the box, where it is safe from trauma.

At the five- and ten-cent store a handle for old razor blades may be purchased; this is a very useful instrument.

Many cut surfaces do not lend themselves well to mounting because later, on rough handling they shed bits of tissue, as does, for example, the cut surface of a tuberculous lung with advanced necrosis and caseation. To prevent this later crumbling we dip or paint the slab of tissue with a hot solution of 4 per cent agar before tying it to the celluloid mount. Formalinized gelatin may also be used.

CONCLUSION

There is presented a method of making specimen containers of sheet glass top and bottom and side walls of glass, held together by asphalt. No metal is used. Very shallow boxes for showing plane surfaces of sliced tissue are simple and effective; deeper boxes to show contour may be made by a modification of the standard method.

REFERENCES

1. Davis, J. E., and Amlosh, A. L.: Preservation of Thin Sections of Tissue in Natural Colors, Arch. Path. **14**: 372, 1932.
2. Aronson, William, and Taub, Jacob: Economical Method of Demonstrating Thin Sections of Tissue, Arch. Path. **21**: 227, 1936.
3. Robertson, H. E., and Lundquist, R.: Preparation of Pathologic Specimens for Exhibition Purposes, Bull. Internat. Assn. Med. Museums **13**: 31, 1934.

"SKIN PRINTS"*

A Simple Technique for Following Individual Lesions in Chronic Skin Disease Such as Psoriasis

Joseph Krafka, Jr., M.D., Augusta, Ga.

IN FOLLOWING the response of psoriasis to viosterol treatment,[1] it became necessary to develop a technique whereby specific lesions could be compared week by week. It is impossible to carry over in the mind the state of a given psoriatic patch for any length of time, and it is entirely too expensive to photograph each patch each week. Hence, the system of "tongue prints" used in connection with the study of pellagra has been adapted to meet the present needs.

Fig. 1.—Three skin prints made on lesions on the wrist of patient: Nov. 6, Nov. 30, and Dec. 17, 1936.

The necessary equipment and the technique are simple. A sheet of thin gloss paper (such as kymograph paper) is smoked in the flame of a gas jet or candle. The smoked paper is applied to the lesion and gently rubbed. It is then removed and shellacked.

A week-to-week record may thus be made as a part of the permanent history of the patient and the efficacy of a given treatment may thus be established.

REFERENCE

1. Krafka, Joseph, Jr.: A Simple Treatment for Psoriasis, J. Lab. & Clin. Med. **21**: 1147, 1936.

*From the University of Georgia School of Medicine.
Received for publication, February 1, 1937.

A COMPARISON OF A SERIES OF WASSERMANN AND KLINE TESTS WITH RESPECT TO SPECIFICITY AND SENSITIVITY*

RALPH G. STILLMAN, NEW YORK, N. Y.

AN ACCURATE evaluation of the comparative reliability of the numerous serologic tests which have been proposed for the diagnosis of syphilis is extremely difficult if not impossible of accomplishment. The technical procedures involved are susceptible of disturbance by the idiosynerasies of the operators so as often to nullify an essential superiority of method and in consequence the results of a comparative study must be interpreted with full realization of the existing technical situation. Three comparative studies have been undertaken under the auspices of the League of Nations, twice in Copenhagen and once in Montevideo, in which serologists from various parts of the world traveled to the place of conference and there carried out their examinations. Valuable information has been obtained from these studies though the number of methods compared has not included several that are quite generally used in this country. In the early part of 1935 a similar study was carried out in this country under the auspices of the United States Public Health Service and the American Society of Clinical Pathologists in which each serologist remained in his own laboratory and the specimens of blood and spinal fluid were sent to him. Each method was carried out by the author except that when two techniques had been devised by one author, the second was carried out by another serologist designated by him. The technical conditions thus approached the ideal. The results obtained with serum specimens are graphically shown in two charts for the use of which I am indebted to Dr. B. S. Kline of Cleveland.

These charts are based upon the examination of about 415 serums from syphilitic patients and about 467 serums from individuals in whom the diagnosis of syphilis had been excluded as far as possible. In Chart 1 the thirteen methods are ranked in the order of their sensitivity. The positive results are shown in black and the doubtful or weakly positive in outline. Positive and doubtful results occurring in the nonsyphilitic group are called "false positives" and "false doubtfuls." It is seen that the 3 most sensitive of the group yielding less than 1 per cent false positives and 7 of the 8 leading tests taking both groups together, are flocculation tests. This greater sensitiveness of the flocculation technic has long been recognized. It is seen that the Kline exclusion test is the most sensitive of those yielding less than 1 per cent false positives but yields a large percentage of false doubtfuls. The Kahn standard test is second and the Kline diagnostic test third. The Hinton test is also exceedingly sensitive but shows more than 1 per cent false positives.

*From the Central Laboratories, New York Hospital and the Department of Applied Pathology and Bacteriology, Cornell University Medical College.

Received for publication, February 4, 1937.

Read before the Cornell University Medical Research Society, November 9, 1936.

In Chart 2 the methods are ranked in the order of their specificity. Again we find three flocculation tests leading and this may create surprise, since it has been rather generally believed that the flocculation technics are so sensitive that they are more likely to yield false positives than are the complement-fixation

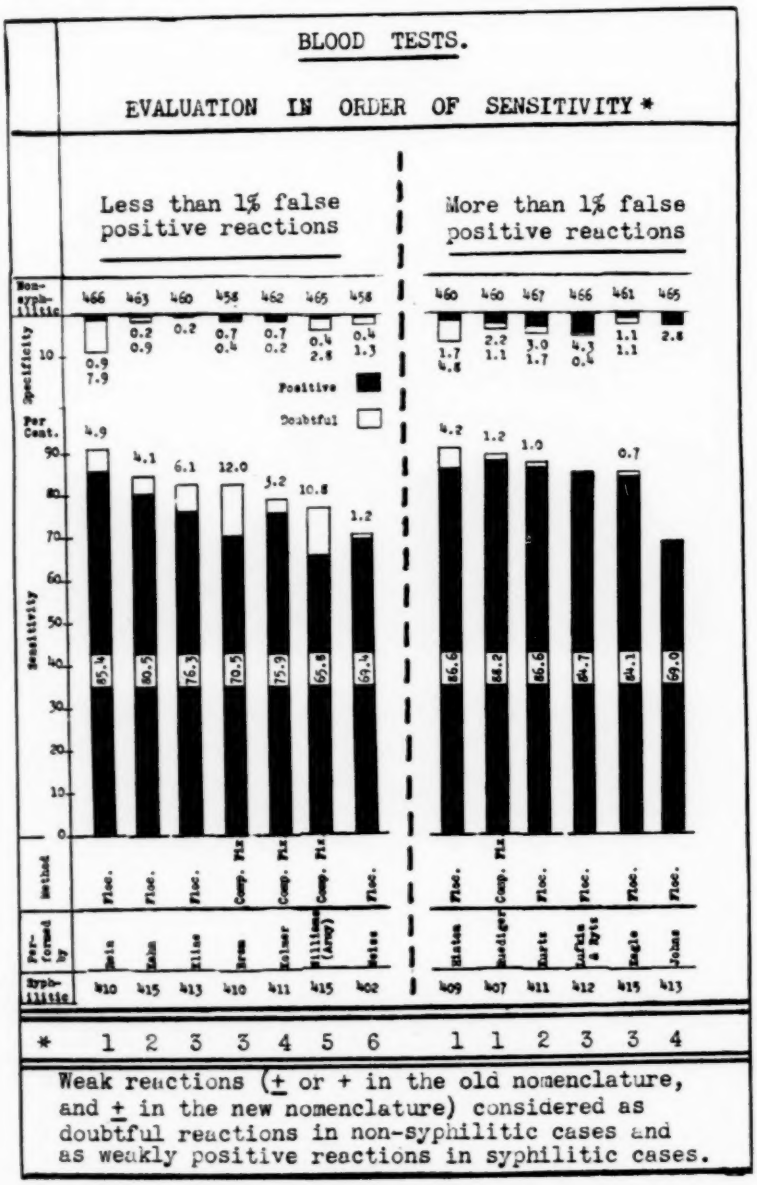

BLOOD TESTS.
EVALUATION IN ORDER OF SENSITIVITY *
Less than 1% false positive reactions
More than 1% false positive reactions
Non-syphilitic
Specificity
Per Cent.
Positive
Doubtful
Sensitivity
Method
Performed by
Syphilitic
Floc. Floc. Floc. Comp. Fix Comp. Fix Comp. Fix Floc.
Weis Kahn Kline Brem Kolmer Williams (Army) Weiss
Hinton Boedger Hurtz Lufkin & Ayer Eagle Johns
* 1 2 3 3 4 5 6 1 1 2 3 3 4
Weak reactions (± or + in the old nomenclature, and ± in the new nomenclature) considered as doubtful reactions in non-syphilitic cases and as weakly positive reactions in syphilitic cases.

Chart 1.—Ranking of the tests in the order of their sensitivity.

tests. It is seen here that the Kline diagnostic test yields but 0.2 per cent false positives and the Kahn and Weiss tests are not far behind. The Kline exclusion test occupies seventh place in this ranking, since it shows 0.9 per cent false positives and 7.9 per cent false doubtfuls.

It must be recognized, as we have said, that these tests were carried out under practically ideal technical conditions. It is not likely that similar results will be obtained when tests are carried out in large numbers as a routine procedure by a group of technicians. Nevertheless, it is an interesting

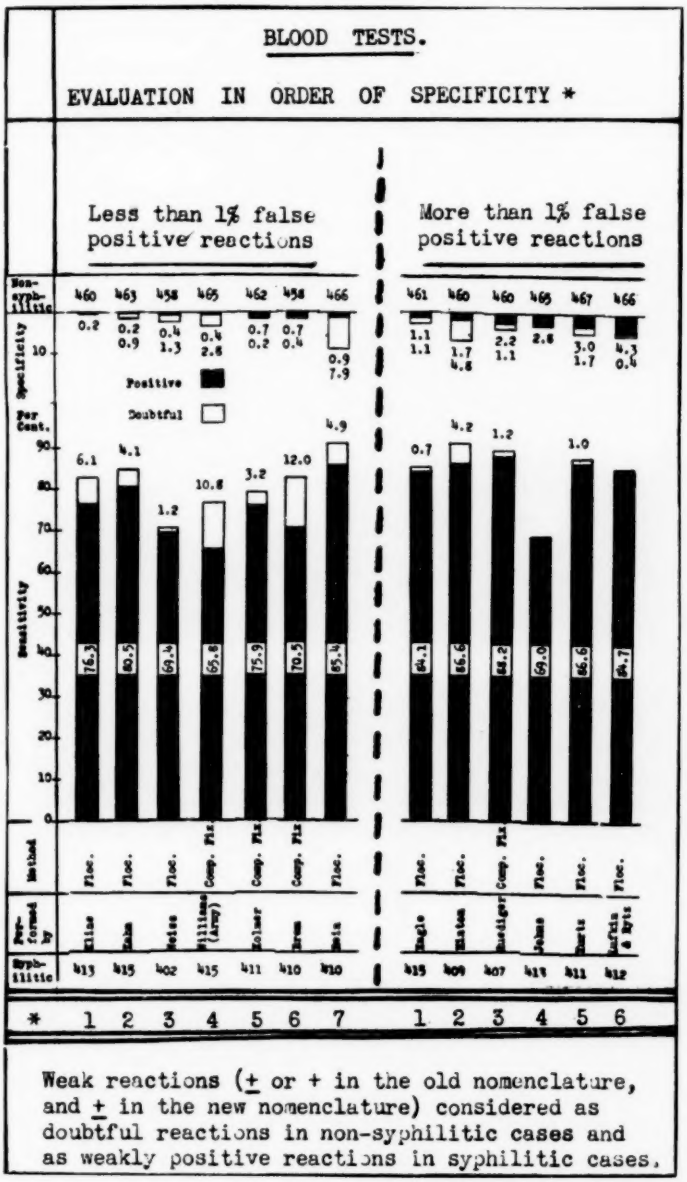


Chart 2.—Ranking of the tests in the order of their specificity.

commentary on the excellence attained by these serologic procedures, that when the results with two or more methods are in agreement and are repeatedly strongly positive, there is usually little doubt that the patient has syphilis. In other words, we accept them as clinically specific though we

realize they are nonspecific when considered as immunologic reactions. In the above study only two conditions showed consistently a significant number of false positive reactions. In 50 patients with leprosy, presumably free from syphilis, about 65 per cent gave positive results and in 36 cases of malaria there were about 15 per cent false positive reactions. In 53 cases of active tuberculosis 2 per cent to 5 per cent of the tests are regarded as false positives while in small series of serums from patients with malignant disease, jaundice, fever, pregnancy, and during menstruation the number of false positives was not increased. The results obtained in leprosy have been criticized on the ground that Larsen in Hawaii found the percentage of positive results he obtained in lepers to be approximately the same as he obtained in the general population from which the lepers were derived. No information of this nature is available for the donors of the specimens examined in this conference but it is stated that each individual giving a false positive result was carefully restudied clinically in order to exclude syphilis as far as possible.

When the results obtained by different methods are widely different, the clinical interpretation becomes especially difficult. In order to obtain some information concerning the significance of such findings as obtained with the technic applied at the New York Hospital, we have examined the charts of a series of patients whose blood has shown a four-plus Kline diagnostic test and a negative Wassermann test. The technic of the Wassermann test used was that recommended by Wadsworth and followed by the New York State Department of Health. As far as possible conclusions have been based upon findings definitely recorded in the history but not infrequently it has been necessary to accept an opinion expressed by the physician in charge of the patient at the time without the support of definite evidence in the record. In this series we have not found in any instance a specimen which yields either a weakly or strongly positive Wassermann reaction when the Kline test was completely negative, although it was not very uncommon to find the Wassermann somewhat more positive than the Kline. This is said to happen especially with blood from the umbilical vein, although we have not seen such results. No attempt has been made in this series to detect or review instances in which the Wassermann test has yielded false positive results.

During the period of a little more than six months, from Jan. 1 to about July 10, 1936, there were submitted to the laboratory 9,358 specimens of blood for serologic tests for syphilis. In 154 of these, derived from 125 patients, the Kline diagnostic test was four-plus and the Wassermann test was negative with both the acetone-insoluble and cholesterolized antigens. This is an apparent patient-incidence of 1.33 per cent, though in reality somewhat higher because of a certain amount of repetition of examinations in the other patients. Of these 125 patients, two were prospective donors of whom no history was available, one was a patient of a laboratory outside of the hospital, one was seen in the Accident Ward and the history was necessarily incomplete and in one instance the record was not found. The histories of the remaining 120 patients were examined in detail (see Table I).

In 79 (65.8 per cent) of these cases in which there was a positive Kline diagnostic test and a negative Wassermann, a diagnosis of syphilis was re-

garded as certain or justifiable. All of these patients had given a positive Wassermann test at some time. In 21 (17.5 per cent), the presence of syphilitic infection was considered to be uncertain, while in 20 (16.6 per cent) there appeared to be no good reason to suspect syphilis except for the result of the Kline test.

TABLE I

TABULATION OF THE FINDINGS IN THIS REVIEW

Positive cases:			
Without history	21		
With history	42		
Congenital syphilis	7		
"Serologic" syphilis	9		
Total positive cases		79	(65.8%)
Doubtful cases:			
With suggestive findings	18		
Probably negative	3		
Total doubtful cases		21	(17.5%)
Negative cases:			
Pregnancy	6		
Infection	4		
Allergy	2		
Diabetes	1		
Laboratory error (?)	1		
Scanty information	6		
Total negative cases		20	(16.6%)
Total records examined		120	

In the table it will be seen that the 79 positive cases fall into four groups: (1) Twenty-one patients definitely denied a history of an initial lesion, but many of them exhibited characteristic lesions such as syphilitic ulcer of the palate, papular syphilide or vascular syphilis; some admitted that the spouse had a positive history and was under treatment; 2 had children in whom a diagnosis of syphilis had been made and in 2 an examination of the spinal fluid was positive. (2) In 42 there was a positive history of syphilitic infection. A number of these were under treatment at the time of their admission to this hospital, and it was necessary to rely largely upon the diagnosis made in other institutions and upon the statement of the patient. In 9 of this group, the spinal fluid examination was positive and the patients presented symptoms of neurosyphilis. (3) In 7 patients the infection was apparently congenital, all of them presenting a definite history. Several of this group suffered from interstitial keratitis. (4) Finally, in 9 patients the diagnosis was made of "serologic syphilis" there being but little tangible evidence of syphilitic infection other than that furnished by the serologic tests. All of these patients had received treatment so that the results obtained with the serologic tests demonstrate the fact which has been observed elsewhere that in syphilitic patients under treatment, the Kline test frequently remains positive after the Wassermann has become negative.

Of the 21 patients classified as doubtful, in whom it was believed by the attending physician that the diagnosis was not certain, there were 18 in whom

there was historical or clinical evidence suggesting syphilitic infection. These patients presented no history of having had a positive Wassermann. Eight patients had had miscarriages, 1 had given birth to a macerated fetus, 3 had had genital sores, 2 had been told at some time they had syphilis, in 1 the Kahn test was also positive, 2 had received antisyphilitic treatment in other hospitals, the father of one is said to have died of syphilis, and 5 were suffering from some type of vascular disease which was possibly syphilitic in origin. In 3 cases there was no evidence pointing toward syphilis.

Among the 20 cases that were regarded as negative there were 6 pregnant women, 4 cases of serious infection (lung abscess, subacute bacterial endocarditis, acute mastoiditis and lobar pneumonia), 2 patients with allergic manifestations, 1 with diabetes without acidosis, and 1 instance probably of laboratory error since the subsequent Kline tests were negative. The remaining 6 charts lacked sufficient discussion of the probability of syphilis to give the reviewer more than an impression that the physician regarded the patient as nonsyphilitic. Several of these patients were seen only once. It will be noted that this group of false positives shows a high incidence of pregnant women which is contrary to the experience of the recent serologic conference. It is not possible to reconcile these results, but it should be stated that the sources of our specimens include an active obstetric service so that the proportion of pregnant women is probably fairly large.

If one adds to the 20 cases regarded as definitely nonsyphilitic, the three from the doubtful group that were probably free from the disease, one finds in these 9,358 consecutive tests 23 (0.24 per cent) strongly positive Kline tests which are probably false positives. The true incidence is higher because no allowance has been made for the repetition of tests on the same patient and the figure given is a percentage of the total number of tests not of the number of nonsyphilitic patients. However, the correction to be made because of these factors may be estimated by deducting from the total number of tests one-sixth for repetition (the experience in the 154 tests reviewed) and then 10 per cent as possibly syphilitic. Thus adjusted the percentage of falsely strongly positive results appears as 0.32 per cent.

Since the Kline exclusion test is more sensitive than the diagnostic test and yields a comparatively large number of false doubtful reactions, it may be objected that its use merely adds to the confusion where a number of methods are reported. However, there is a practical advantage. Those specimens which yield a negative result with both the exclusion and the diagnostic tests may safely be regarded as serologically negative and by carrying out Wassermann tests only on those specimens which yield more or less positive results with the flocculation technic we are saved the time and expense of performing many complement-fixation tests and will only rarely miss a positive Wassermann.

In reviewing these records it was frequently noted that when a four-plus Kline and a negative Wassermann were obtained in old syphilitics, the results were likely to be the same on several successive examinations. On the other hand, in those patients who were presumably nonsyphilitic, repetition of the test was likely to yield less strongly positive results and the results tended to

vary from time to time. For these reasons and to exclude the possibility of certain errors either in the laboratory or in the clinic, repetition of the examination should always be requested when the result is equivocal. Such "repeat examinations" are not requested as freely as is desirable, sometimes of course because the patient does not return to the clinic for further study.

It may be concluded that as carried out by a group of technicians as a routine procedure in this institution, the Kline test is more sensitive than the Wassermann and that this sensitivity is attained without sacrifice of an appreciable amount of specificity; also that on the whole serologic tests for syphilis seem to have reached a rather high degree of clinical specificity. From the figures it appears that when we report a four-plus Kline and a negative Wassermann, the odds are at least two to one that the patient has a syphilitic infection and that if one-half the doubtful cases are regarded as positive the odds rise to three to one. Therefore, when such a report is made, the presumption is created that the patient has syphilis, and it becomes incumbent upon the clinician to exclude this possibility so far as that can be done. The contrary result, a negative Kline test and a positive Wassermann is known to occur at times but from our experience in the present series, it may be said that if the Kline test is wholly negative, it is unlikely that another method will yield anything but a negative result.

AN EASILY CONSTRUCTED RESPIRATORY VALVE*†

R. H. K. Foster, M.D., Ph.D., Nutley, N. J.

THIS description is presented with the view in mind of illustrating an efficient respiratory valve easily constructed with ordinary laboratory materials. The apparatus as described may be used for two animals simultaneously, or for one alone. The dimensions given make it most suited to small dogs, cats, and rabbits. For smaller animals the tubing leading from the animal to the expiratory valve must be of smaller bore than indicated. For large dogs, all dimensions must naturally be increased. When two animals are anesthetized with the apparatus, they have the same degree of anesthesia since the ether vapor concentration is controlled by only one ether bottle. Although this might be an objection in some experiments it has been found that with light anesthesia there is little difference in the corneal reflexes or gastrocnemius tonus of the two animals.

This respiratory valve is particularly well adapted for use with the ether bottle described in a previous communication.[1] A connection is made from the ether bottle to the T-tube, *A* (Fig. 1). All rubber tubing is ¼ inch I.D. and glass tubing 8 mm. O.D. During inspiration, air enters *A*, passes through the inspiratory valves, along tube *B*, and exits to the animal. During expiration, air passes from the animal into bottle *C*, and out through the expiratory

*Received for publication, February 23, 1937.
†From the Pharmacological Laboratory of Hoffmann-La Roche, Inc.

valve. The valves themselves are made from rubber dam, cut as shown, and fastened to the stoppers with rubber cement. The inspiratory valves are made by fitting No. 10 rubber stoppers into 2-inch Stender dishes. The Stender dishes may be supported separately or, for convenience, fastened together with adhesive as shown, and supported by a clamp. *C* is a wide mouth specimen bottle, 2 inches in diameter and 4 inches in height, taking a No. 9 rubber stopper.

If the ether vapor concentration is not the same on the two sides of the thin, rubber dam valves, they tend to curl upward. Therefore to maintain an ether vapor atmosphere above the outlet valves, a short section of 1-inch glass tubing is placed over the valve. It should be held in place by copper wire fastened to the T-tube.

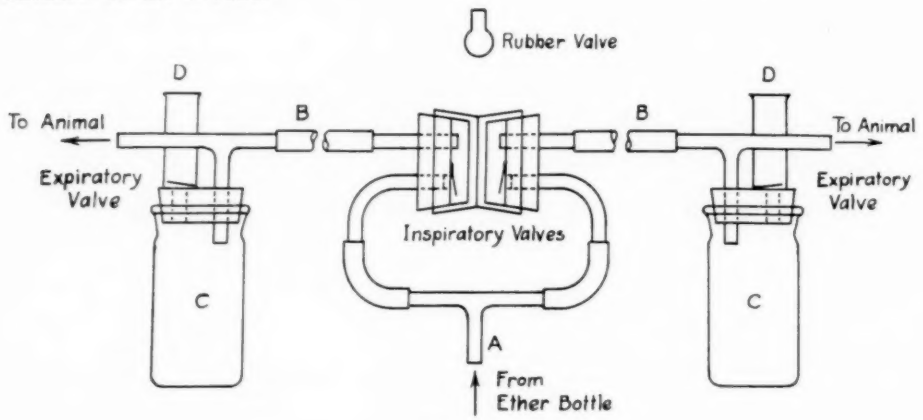


Fig. 1.

The connection from the expiratory valve to the animal must be as short as possible to minimize the dead space in the common passageway for inhalation and exhalation. Tube *B* may be as long as desired since only inspired air passes through it. Stender dishes instead of bottles are used for the inspiratory valves since a large dead space here would lengthen unnecessarily the time lag whenever it is desired to alter the depth of anesthesia. Although the actual size of bottle *C* is immaterial, the size indicated was found most convenient as the bottle must be placed as close as possible to the tracheal cannula. The rubber dam on the valves must be renewed every three or four months: a comparatively simple matter.

REFERENCE

1. Foster, R. H. K.: An Improved Ether Bottle for Animal Anesthesia, J. LAB. & CLIN. MED. **17**: 490, 1932.

THE MEASUREMENT OF FLUORESCENCE INTENSITY BY PHOTO-ELECTRIC MEANS*

DANIEL S. STEVENS, PH.D., AND WILLIAM J. TURNER, M.D., CHICAGO, ILL.

IN THE course of some work on Porphyria it seemed desirable to carry out some quantitative porphyrin determinations. A review of the literature revealed that there are in general only two methods which satisfy both practical and theoretical requirements of an exact determination. The one, based on the extinction coefficients of porphyrins at various wave lengths, requires an expensive spectrophotometer as well as considerable experience and time.[1] The other, based on the intensity of the red fluorescence of porphyrins in ultraviolet light, requires a photometer of the König-Martens or Pulfrich type.[2] Since this was not at hand it was thought worth while determining whether a photo-electric circuit could be set up which could measure the faint fluorescence of dilute solutions of porphyrins, and yet be both inexpensive and readily reproducible.

The apparatus described below fulfills these requirements. It has not been possible to extend our observations to precise quantitative determinations through lack of a constant source of ultraviolet light.

Apparatus (Fig. 1).—The source of ultraviolet used is an arc between National "C" carbons, 13 mm. diameter, operated at 40 amp., 40 volts, A.C. The arc is surrounded by a water-cooled metal housing in which, directly in front of the arc, there is set a water cell with quartz windows. Between these windows there is placed a Corning glass filter No. 428, 4 mm. thick. Through this the light passes to a round Corning filter No. 586, 4.5 mm. thick set in one side of a copper box. This box has an opening in the top which admits thin-walled Pyrex test tubes, 32 mm. diameter, holding 40 c.c. of solution. Two more openings, at right angles to the former, are fitted with Corning filters No. 348. The filter combination is such that only the rays above 5300 A pass out through the red filters to the shielded photo-electric cells. Thus the porphyrin fluorescence excites the photo-electric current.

The circuit used to measure the intensity of fluorescence is essentially that described by Garman and Droz.[3] Slight modifications introduced to increase the stability and sensitivity of their circuit to our requirements are: increase of grid resistance to 11 megohms; storage battery for the filament supply, and a "B" battery for the plate voltage. The details of the circuit and its balancing are given in the paper cited. It is essential that the entire apparatus be shielded carefully. The photo-current is read on a micro-ammeter, most convenient of which we have found to be a Sensitive Research Multimeter.

Method.—In making a series of determinations, the solutions are made up *immediately* before the start of the run. While one of us prepares the dilutions the other adjusts the arc until it gives a steady flame and then the

*From the Department of Medicine, University of Chicago Clinics.
Received for publication, February 27, 1937.

resistances in the plate circuit until a blank (distilled water) reading remains constant at a suitable low figure on the ammeter scale. Thereupon the solutions to be examined are rapidly inserted in the chamber, alternating fluorescence readings with blanks. An entire run of 20 tubes can be made in three minutes.

We tested the reality of our determinations in two ways: first, by ascertaining that there is a smooth curve relationship between intensity of

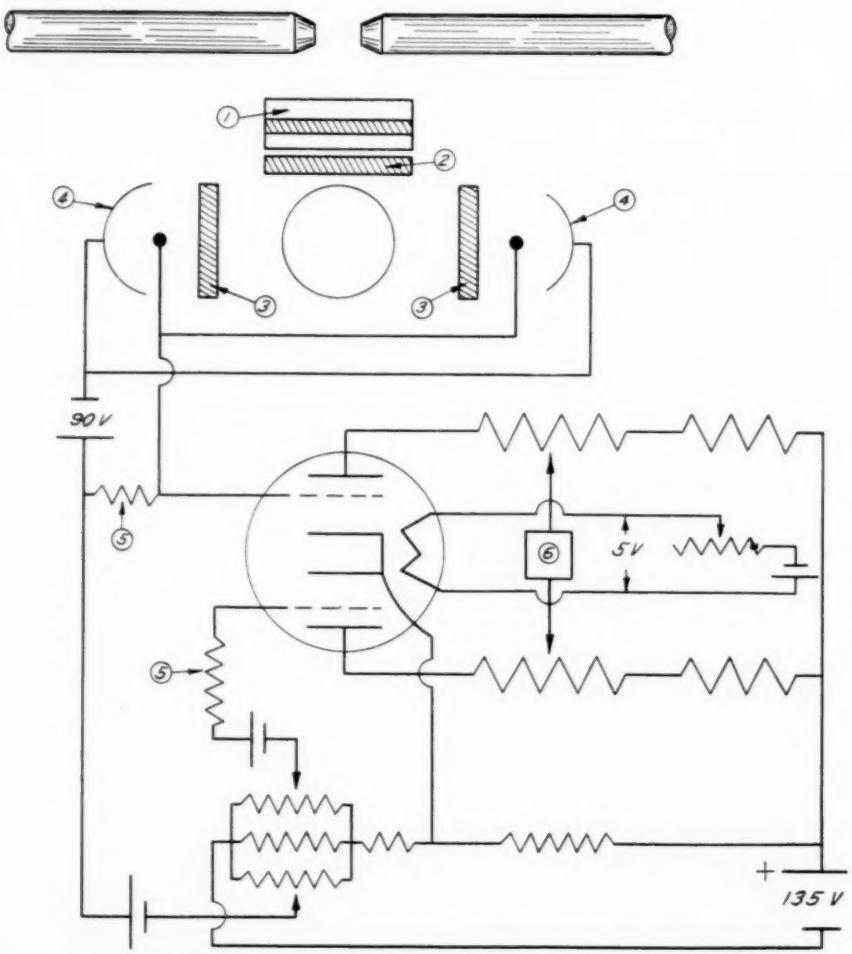


Fig. 1.—Apparatus for measuring fluorescence. (*1*) water cell containing Corning filter No. 428, (*2*) filter No. 586, (*3*)filter No. 348, (*4*) Western Electric photo-cell No. 3A, (*5*) 11 megohms, (*6*) Sen. Res. multimeter. (Other parts of the circuit are as listed in ref.[3]

fluorescence and the logarithm of porphyrin concentration in micrograms per cent (Fig. 2); and second, by demonstrating a smooth curve of change of intensity of fluorescence with change of pH (Fig. 3).

For the former, Uroporphyrin I was dissolved in 2.0 N HCl and diluted in the same solvent in geometric progression. Protoporphyrin IX, dissolved in 0.1 N NH_4OH, and diluted in similar progression, served as a further check.

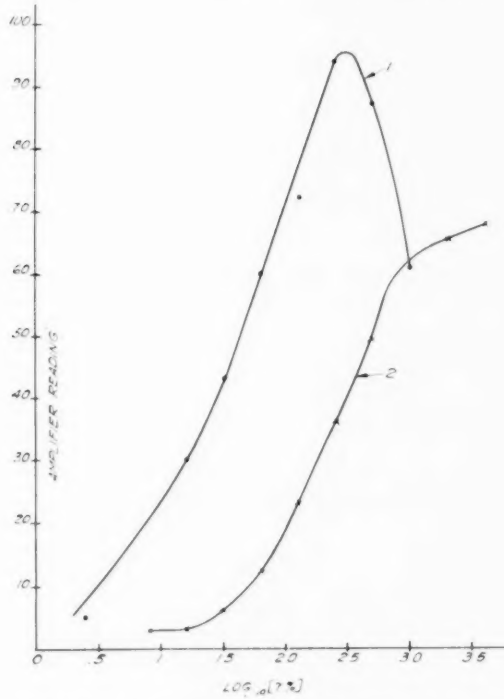


Fig. 2.—Effect of concentration on the intensity of fluorescence of (*1*) protoporphyrin IX in 0.1 N NH_4OH, and (*2*) uroporphyrin I in 2.0 N HCl.

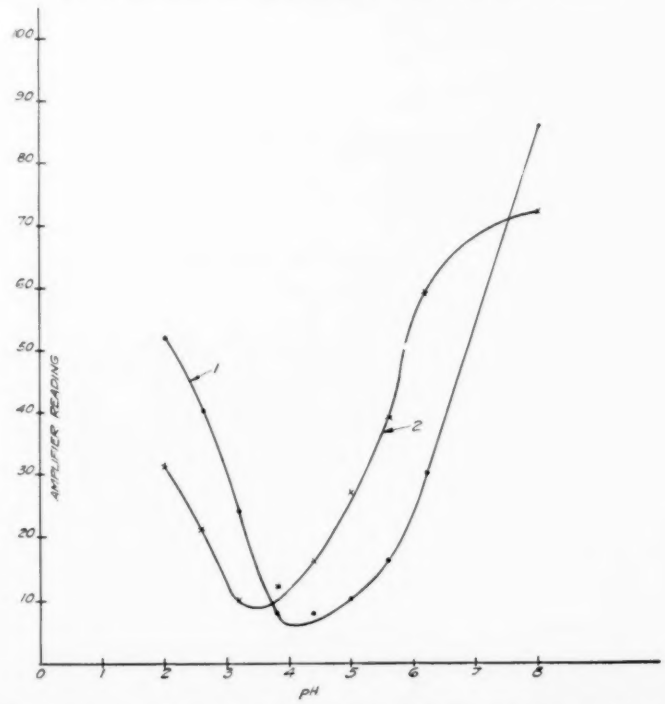


Fig. 3.—Effect of pH on the intensity of fluorescence of (*1*) coproporphyrin I, and (*2*) uroporphyrin I.

In making the pH fluorescence determinations Coproporphyrin I was taken out of ether into 0.40 N HCl. Uroporphyrin I was the same as that used in the dilution tests. Dilutions were made into MacIlvaine's buffer[4] as recommended by Fink and Hoerburger.[5] In the case of Uroporphyrin the final concentration at which the runs were made was 400 γ per cent; of coproporphyrin, about 300 γ per cent.

RESULTS

As demonstrated by Figs. 2 and 3 the method employed is sufficiently sensitive to measure porphyrin concentrations in dilution as low as 10 γ per cent (1 : 10,000,000) and stable enough to compare two porphyrins throughout a range of pHs. The availability of the method for exact and reproducible measurements is limited, we believe, only by the necessity of having a standard, intense and constant source of ultraviolet light. It should be kept in mind that this method can be used for the measurement of fluorescence of any wave length, provided care is taken in the selection of filter combinations.

SUMMARY

A simple, inexpensive method is described whereby the intensity of fluorescence of solutions may be measured by photo-electric means.

REFERENCES

1. Treibs, A.: Eine Methode zur spektrophotometrischen Konzentrationsmessung von Farbstoffen neben gefärbten Begleitsubstanzen, Ztschr. f. physiol. Chem. **168**: 68, 1927.
2. Fikentscher, R.: Quantitative Porphyrin-Bestimmung durch Lumineszenzintensitätsmessung mit dem Stufenphotometer, Biochem. Ztschr. **249**: 257, 1932.
3. Garman, R. L., and Droz, M. E.: Line-Operated Vacuum-tube Voltmeter, Ind. Eng. Chem., Anal. Ed. **7**: 341, 1935.
4. Clark, W. M.: Determination of Hydrogen Ions, Baltimore, 1928, Williams and Wilkins Company.
5. Fink, H., and Hoerburger, W.: Beiträge zur Fluorescenz der Porphyrine, Ztschr. f. physiol. Chem. **218**: 181, 1933.

THE BIOLOGICAL ASSAY OF ESTROGENIC SUBSTANCES*

EVANGELINE F. DECKERT, M.A., ELIZABETH MULHALL, B.A., AND CAROL SWINEY, B.A., JERSEY CITY, N. J.

THE generally accepted method for the biological assay of estrogenic substances is the vaginal smear method. The potency thus determined was originally expressed in rat units or in mouse units, but the values of these units, as well as the ratio of the rat unit to the mouse unit were found to be subject to wide variations. Because of this range of values, the Conference on the Standardization of Sex Hormones of the League of Nations Health Organization in 1933 adopted an International Standard consisting of ketohydroxyestrin and defined the International unit as 0.1 gamma of this standard.

Although the International Conference[1] stated that the International unit was approximately one-third of the Allen and Doisy rat unit, more recent (1934-1936) reports from various laboratories[2-8] have placed the value at from 1/5 to 1/38 of the rat unit. The lack of agreement suggests that the vaginal smear method cannot be used for purposes of standardization with any considerable degree of accuracy. This fact can be appreciated from the divergent views expressed by investigators of the widest experience. Coward and Burn[9] state that the variation in the rat unit or the mouse unit may be as great as 1,000 per cent. In a later publication Bulbring and Burn[10] give results on the determination of the strength of seven different dilutions of a solution of estrone. The maximum error was 30 per cent. In one case where the error was 10 per cent, they present an analysis of the way in which the figure representing the strength of the unknown solution was calculated. In this assay 6 groups of animals were injected with varying doses of the unknown solution and four groups with a known dose of estrone. The number of animals in each group varied from 16 to 23. The same dose of estrone administered to two of the groups gave a response of 30 per cent at one time and 53 per cent at another. In one trial, a given dose of the unknown solution caused a response of 100 per cent, but at another time double this dose produced only a 57 per cent response. The final result was calculated from two closely agreeing determinations, but the earlier discordant results, which were disregarded, introduce a question as to the validity of this result. The details in regard to the determination of the strength of the other six solutions were not given.

The failure of the vaginal smear method to yield approximately accurate results is shown by the relative values of the rat and mouse unit published by various authorities. Coward and Burn[9] state that the average mouse unit and rat unit are equal, while Hain and Robson[7] have found the value of the rat unit to be from 10 to 36 times the value of the mouse unit, depending

*From the Reed and Carnrick Research Laboratories.
Received for publication, February 18, 1937.

upon the conditions of the experiment. Values given by other investigators range between these extremes.[11] It is obvious that such results involve large errors in the determinations of the rat or mouse units in some of the tests.

Differences in technique may be important factors in obtaining such varying results, but in these laboratories where a consistent effort has been made to standardize the technique, the activity of the International Standard from four separate ampules has been tested by the vaginal smear method over a period of three years with widely divergent results. The early results agreed closely with the approximate value given by the International Conference, but at the present time we find a ratio more nearly approaching 1 to 10.

In an attempt to secure a more reliable method for standardizing estrogenic products by comparison with the International Standard, we have used the technique employed by Fluhmann[12, 13] for the estimation of estrogenic substances in the blood of women. This procedure over a period of one year has demonstrated its superiority over the vaginal smear method.

VAGINAL SMEAR TEST ON RATS

Our discussion of the vaginal smear method is based upon results obtained from 128 groups of rats injected with the International Standard. In most cases these groups consisted of twenty rats, although a few groups contained less and a number of groups more than 20 animals. The following procedure, which differs slightly from the method of Kahnt and Doisy,[14] has been used in these experiments.

All animals used are from our own colony in which no change in the breeding stock has been made during the last five years. Rats weighing approximately 120 gm. are subjected to a bilateral ovariectomy, care being taken to excise a small portion of the uterus in order to insure complete removal of the ovarian tissue. After a period of one week, smears are taken daily during the next two weeks, and only rats showing resting stage smears are selected for use. They are kept under standard conditions with particular attention to an adequate diet and are used for assays once in three weeks. Weekly smears and weights are taken on all rats, and only those found in the resting stage are selected for use in assays. The animals are distributed according to age and previous injections so that all groups used at one time are as nearly comparable as possible. If a rat is not used for assay within three weeks, or fails to respond in a previous assay, it is primed with an oil solution of the International Standard. When first used for priming, an injection of 0.6 gamma of the International Standard was given; a dose which, at that time, seemed adequate. Later, however, it was necessary to increase the dose to 0.75 gamma, and at present 1.5 gamma of the International Standard is required as a priming dose. Any rat failing to give a positive reaction to the priming dose is discarded.

Oil solutions of estrogenic substances are injected intramuscularly in a single dose of 0.2 c.c. Smears are first taken approximately seventeen hours later. Aqueous solutions are injected subcutaneously in three equal doses at three and one-half hour intervals, the total dose being 0.6 c.c. The vaginal

smears are first taken seventeen hours after the last injection. The smearing is continued twice daily for three days following injection, although in some of the earlier work smears were taken three times daily. To obtain the smear, a glass rod, about 2.5 mm. in diameter, slightly drawn out and carefully rounded at the end, is inserted and rotated in the vaginal canal and the material on the rod is transferred to a drop of distilled water on a glass slide. The wet smears are examined microscopically and are then frequently dried and stained with Harris' hematoxylin-eosin to confirm the reading of the wet smears.

The resting stage is characterized by the presence of a large number of leucocytes occurring alone or with a very small number of nucleated or cornified cells. The reaction smear is one in which practically only cornified cells are present (strongly positive), or one in which the cornified and nucleated cells have increased markedly while the leucocytes have definitely decreased (threshold reaction).

The following tables give the results obtained in testing four separate ampules of the International Standard. These ampules are designated as:

A: Received Feb. 21, 1934.
Approximately 1.0 mg. portions of the standard were weighed on a microbalance and stored in a vacuum desiccator over phosphorus pentoxide. Solutions were prepared immediately before using.
B: Received Dec. 14, 1934.
C: Received Dec. 27, 1935.
D: Received Dec. 14, 1936.
The contents of B, C, and D were weighed, made up in alcoholic solution and stored in tightly stoppered bottles at about 3° C. Aliquot portions were used to prepare the solutions for injection at the time of assay.

In the tables, the number of animals reacting positively is shown as a percentage of the entire group. Each group is made up of 20 rats except where indicated parenthetically after the reaction percentage.

TABLE I

INTERNATIONAL STANDARD (A)

DATE	AQUEOUS SOLUTIONS			OIL SOLUTION
	0.3 GAMMA	0.45 GAMMA	0.6 GAMMA	0.3 GAMMA
3/16/34	60%(25)	60%(25)		
5/11/34	84%(25)	72%(25)	76%(25)	
12/21/34	80%(25)	76%(25)		
3/4/35				80%(25)
8/29/35				55%
12/13/35	15%			
12/30/35				30%

An examination of the tables shows a wide variation in the response of different groups on any given dosage, as well as many instances where the response from a larger dose is no greater or is even less than from a much smaller dose. Since doses that formerly produced satisfactory responses have not been effective in our later work, it might be assumed that the International Standard was subject to deterioration, but in Table II where the same alcoholic solution was used over a period of thirteen months, there is no regularly

decreasing response to corresponding doses. This is in agreement with the results obtained by Rowlands and Callow.[15] Individual variation among the animals is an important factor in this irregularity of results. This is shown

TABLE II

INTERNATIONAL STANDARD (B)

	AQUEOUS SOLUTIONS		OIL SOLUTIONS			
DATE	0.3 GAMMA	0.6 GAMMA	0.3 GAMMA	0.6 GAMMA	0.75 GAMMA	1.0 GAMMA
12/19/35	45%		50%			
			50%			
12/30/35			35%			
			15%			
1/9/36			25%		70%	
1/14/36	40%		44%			
1/20/36	10%			65%		54%(37)
						78%(50)
1/27/36					48%(31)	
					73%(33)	
1/31/36		20%				
2/4/36				30%	36%(11)	30%(10)
					67%(27)	43%(14)
					59%(41)	
2/21/36				60%		
2/25/36				60%	26%(19)	
3/3/36				40%	85%(34)	
					47%(15)	
3/11/36				55%	71%(42)	
3/17/36				55%	88%(36)	40%(15)
3/24/36				55%	63%(41)	
				65%		
3/31/36				50%	62%(42)	
4/7/36				40%	73%(33)	
					78%(32)	
4/13/36				30%	58%(38)	
				70%	27%(11)	
4/20/36					64%(42)	
4/29/36		60%		25%		
5/7/36		60%		45%		
5/16/36		85%		30%		
5/26/36					65%	
6/7/36		55%				
6/15/36		50%			59%(22)	
6/23/36		70%			63%(41)	
6/29/36		45%			40%(15)	
					64%(42)	
7/6/36					33%(33)	
7/14/36		65%		55%		
7/21/36				60%		
7/28/36		55%			75%(16)	
					82%(38)	
8/3/36					89%(19)	
					73%(15)	
8/10/36				25%		
8/17/36				35%		
9/8/36		20%				
9/15/36		65%			72%(32)	
9/22/36		40%				
9/28/36				30%	41%(37)	
10/5/36		45%			27%(30)	
10/13/36				45%		
10/19/36	0%					
10/24/36	5%					
10/30/36		65%				
1/18/37						50%

by a study of the rats in Table II, given 0.6 gamma and 0.75 gamma in oil solution. The response of the groups on a dosage of 0.6 gamma varied from 25 per cent to 70 per cent, while on 0.75 gamma, the response was from 27

TABLE III

INTERNATIONAL STANDARD (C)

DATE	AQUEOUS SOLUTIONS			OIL SOLUTIONS				
	0.6 GAMMA	0.75 GAMMA	1.0 GAMMA	0.3 GAMMA	0.6 GAMMA	0.75 GAMMA	1.0 GAMMA	1.5 GAMMA
11/16/36					15%			
11/22/36					20%			
12/ 1/36	15%							
12/ 7/36						25%		
12/15/36		30%						68% (34)
12/21/36						40%		77% (40)
								95% (42)
1/ 4/37								80% (20)
								47% (15)
								50% (16)
1/18/37							60%	
1/25/37						25%		
2/ 2/37			35%					
			45%					

TABLE IV

INTERNATIONAL STANDARD (D)

DATE	AQUEOUS SOLUTIONS			OIL SOLUTIONS				
	0.6 GAMMA	0.75 GAMMA	1.0 GAMMA	0.3 GAMMA	0.6 GAMMA	0.75 GAMMA	1.0 GAMMA	1.5 GAMMA
12/28/36		0%						
1/ 5/37		5%						
1/12/37								94% (18)
								50% (14)
								81% (16)
								71% (21)
1/18/37							40%	95% (18)
								50% (14)
								81% (16)
								71% (21)
1/25/37								55% (11)
								73% (30)
2/ 2/37			60%					78% (27)
								75% (8)

TABLE V

CLASS I—REGULAR RESPONSES			CLASS II—IRREGULAR			CLASS III—NEGATIVE RESPONSES		
DATE	DOSE IN GAMMA	RESULT	DATE	DOSE IN GAMMA	RESULT	DATE	DOSE IN GAMMA	RESULT
1/20/36	0.60	–	12/30/35	0.30	+, 3rd A.M.	12/19/35	0.3	–
1/27/36	0.75	+, 2nd A.M.	1/14/36	0.75	–	1/6/36	0.75	–
2/25/36	0.75	+, 2nd P.M.	1/20/36	0.75	–	1/13/36	1.00	–
3/9/36	0.60	–	2/4/36	0.75	+, 3rd A.M.	1/27/36	0.75	–
3/17/36	0.75	+, 1st A.M.	3/3/36	0.75	+, 1st A.M.	2/3/36	1.00	–
4/6/36	0.60	–	4/20/36	0.75	–			
4/12/36	0.75	+, 2nd A.M.	4/27/36	0.75	–			
5/5/36	0.60	–	5/25/36	0.75	–			
5/11/36	0.75	+, 2nd A.M.	6/1/36	0.75	+, 2nd P.M.			
			6/29/36	0.60	+, 2nd P.M.			
			7/20/36	0.75	–			
			8/3/36	0.75	+, 3rd A.M.			

per cent to 89 per cent; thus the highest response from 0.6 gamma was more than two and a half times as great as the lowest response from 0.75 gamma. The average response of the 440 animals at 0.6 gamma was 46.6 per cent, and of the 908 animals at 0.75 gamma, 60.6 per cent. These figures are roughly proportional to the doses and suggest that, if accurate results are to be obtained, a very large number of animals must be used. In order to determine whether rats differed among themselves in the amount of standard required to produce oestrus or whether the same rat required different doses at various times, the records of 100 rats, each injected five or more times with Standard B, were examined. These animals could be divided into three groups:

I. 15 animals that responded regularly to the same doses.
II. 78 animals that responded irregularly to the same doses.
III. 7 animals that were always negative to the doses administered.

Table V shows the records of a typical animal from each of these groups.

The largest factor, therefore, in the irregular responses of groups of rats seems to be the varying dose required to produce a positive smear in the same rat at different times, and no method of selection or standardization of animals can correct the error due to a fluctuating sensitivity of a rat to estrogenic substances.

It has been suggested that groups of rats of approximately the same age and weight and ovariectomized on the same day will respond more uniformly to estrogenic materials than miscellaneous groups. To test this point, twenty animals, born Oct. 11 and 12, 1936, were ovariectomized Dec. 4, 1936. Each time these animals were used, they showed as great irregularities as rats of differing ages and periods of use selected in the ordinary way from our ovariectomized colony. As an example, on Feb. 1, 1937, a dose of 1.0 gamma was administered to this group, with the result that eleven gave a negative and nine a positive reaction, thus showing that in this case practical identity in age and time of ovariectomy contributed nothing to the uniformity of response.

The method depends upon the examination of a vaginal smear to determine the condition of the vaginal mucosa, and if this is not always correctly interpreted by the character of the smear, a large source of error will be introduced. In investigating the influence of this possibility, smears were taken from 11 control rats and 43 rats injected with the International Standard. The injected rats were killed as soon as they showed a positive smear. Those that failed to react positively during the three days following injection

TABLE VI

NO. RATS	SMEAR REACTION	TISSUE REACTION
5	Strongly positive	5 Positive (6 to 10 layers epithelial cells with cornified layer at surface.)
5	Threshold	3 Positive
		2 Weakly positive (4 to 8 layers of cells, no cornified layer.)
33	Negative	13 Weakly positive
		20 Negative (2 to 4 layers epithelial cells.)

were then killed together with the controls. The vaginal tissue was removed and sections prepared for microscopic examination.

In the control group, 7 of the 11 smears showed a preponderance of leucocytes with a variable number of nucleated and cornified cells. In the 4 other smears, the nucleated cells far outnumbered the leucocytes. In spite of the variation in the control smears, the tissue of all 11 rats proved to be of the castrate type, with only 2 to 4 layers of epithelial cells. The vaginal smears from four of the control rats, therefore, failed to show accurately the

Fig. 1.—Smear, Rat 6144.

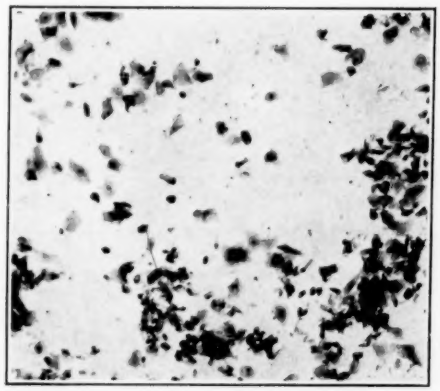


Fig. 2.—Smear, Rat 6144.

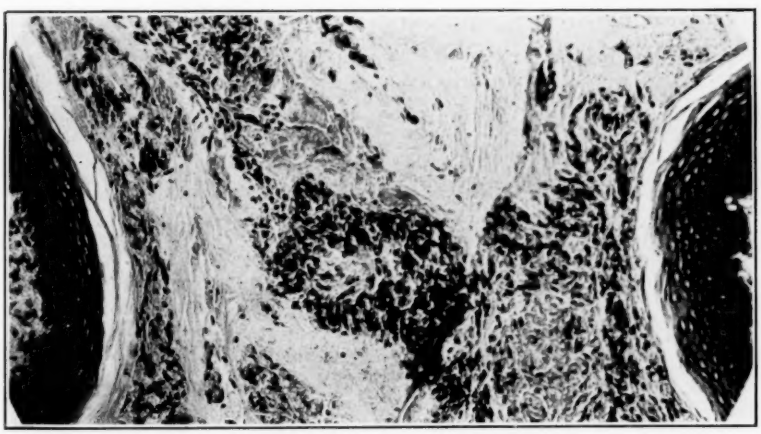


Fig. 3.—Tissue, Rat 6144.

condition of the vaginal mucosa. This fact is of importance since rats showing smears containing chiefly nucleated cells are not considered suitable for use in assays.

In the injected group, the smears and tissue reactions, as shown in Table VI, were obtained.

As a rule, when a strongly positive smear was obtained, the tissue showed marked stratification with cornified cells at the surface. But with the threshold and negative smears, it was more difficult to correlate the smear with the state of the vaginal tissue. For example, in the smear from Rat

6144, two fields from which are shown in Fig. 1 and Fig. 2, the number of leucocytes, occurring with many cornified and nucleated cells, was so great that the rating as a positive smear might be questioned, but the examination of the tissue (Fig. 3) left no doubt that the rat had responded to the injected dose.

On the other hand, the smear from Rat 6061 (Fig. 4) contained so many cornified cells that it might be considered a threshold reaction, but the tissue proved to be castrate in type (Fig. 5). The appearance of such large numbers of cornified cells in a smear from a rat which did not attain estrus may be explained by the contamination of the smear with desquamating cornified cells, characteristic of protective epithelium, which are seen in sections of the vaginal tissue near the external orifice.

The above results, obtained on a limited number of animals, indicate that in ovariectomized rats, the type of smear does not necessarily reveal the condition of the vaginal tissue, but more work is required to determine how large an error is introduced in the vaginal smear method by this factor.

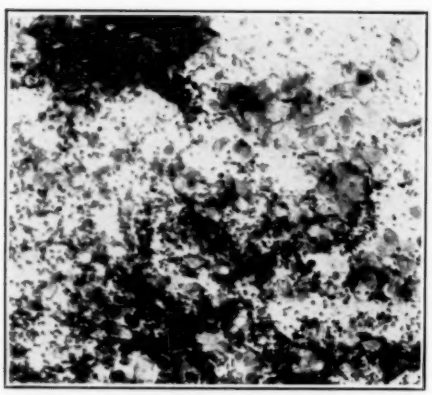


Fig. 4.—Smear, Rat 6061.

Fig. 5.—Tissue, Rat 6061.

Another serious weakness in the vaginal smear method is the personal error which may occur in interpreting smears of the threshold type, where judgment of the stage of reaction is dependent upon the worker's impression of the ratios of the various types of cells.

MUCIFICATION TEST ON MICE

Fluhmann's histologic test[12, 13] for the presence of estrin in the blood of women depends upon proliferation of the epithelium in the vaginal mucosa of recently castrated mice, and the formation of mucin cells. His method, in brief, is as follows:

A definite amount of blood serum is injected subcutaneously into six- or seven-day castrate mice over a period of three days, three injections being given per day: at 8 A.M., NOON, and 5 P.M. On the fourth day, the mice are killed, the vaginas dissected free and preserved in formalin, and paraffin sections are made. Transverse sections are made at different levels, and the sections are stained with hematoxylin-eosin.

Fluhmann recognizes six reactions as follows:

Reaction 0: Atrophy of the vagina; two layers of low cuboidal epithelium. Occasional leucocyte.

Reaction 1: Mucosa shows two layers, a basal of low cuboidal epithelium and a superficial of tall columnar cells. A few leucocytes.

Reaction 2: Superficial cells are high, show stratification and secrete mucus. Marked increase in leucocytes which may appear in lumen.

Reaction 3: Epithelium composed of several layers with the surface cells of the mucified variety. Infolding of the mucosa. The mucosa is invaded by large numbers of leucocytes which occur also with epithelial debris in the lumen.

Reaction 4: The mucosa made up of from 6 to 12 rows of cells. Lower resembling basal cells of squamous epithelium, while surface cells are of the mucified variety. Cornification may be observed between the stratified epithelium and the mucified cells. Few or no leucocytes.

Reaction 5: Vagina lined with squamous epithelium with cornified layers at surface. No leucocytes.

Following Fluhmann's procedure as closely as possible, the International Standard B was first injected in aqueous solution. At the time this work was begun, assays were being run by the vaginal smear method on rats, using the International Standard at 0.75 gamma and at 0.3 gamma, and it was decided to inject these same amounts into the mice. Both amounts were put into solution so that the total dose injected per mouse was 0.9 c.c., the injections being given subcutaneously 0.1 c.c. per mouse three times daily for three days. The mice, three in number for each test, were killed approximately twenty hours after the last injection. The vaginal tissue was removed, fixed in Bouin's, and paraffin sections were made. Transverse sections were cut from the vagina and stained with the hematoxylin-eosin.

When examined microscopically, both the dosages used showed complete cornification of the vaginal epithelium, while the tissue of control castrate mice showed complete atrophy. A second series of tests, using doses of 0.3 gamma and 0.15 gamma, showed almost complete cornification.

The next step was to test the activity of the International Standard in oil solution. Here it was decided to parallel the method of injecting oil solutions in the rat and to use only a single injection, which was given sub-

TABLE VI

INJECTIONS	ROUTE OF INJECTION	TIME OF AUTOPSY	NUMBER OF MICE	REACTIONS
Single	Subcutaneous	72 hr. after injection	3	0,2,0
Divided	Subcutaneous	24 hr. after last injection	2	4,5
Divided	Subcutaneous	48 hr. after last injection	2	5,5
Divided	Subcutaneous	72 hr. after last injection	2	Regression started
Single	Intraperitoneal	24 hr. after injection	2	1,2
Single	Intraperitoneal	48 hr. after injection	2	0,0
Single	Intraperitoneal	72 hr. after injection	2	1,0
Divided	Intraperitoneal	24 hr. after last injection	2	0,0
Divided	Intraperitoneal	48 hr. after last injection	2	1,3
Single	Intramuscular	24 hr. after injection	2	1,1
Single	Intramuscular	48 hr. after injection	2	3,3
Single	Intramuscular	72 hr. after injection	2	3,3
Divided	Intramuscular	24 hr. after last injection	7	4,4,4,5,4,4,5
Divided	Intramuscular	48 hr. after last injection	7	5,5,5,5,5,5,5
Divided	Intramuscular	72 hr. after last injection	3	Regression started

cutaneously. The dose chosen was 0.15 gamma since it had produced positive results in aqueous solution. The mice were killed seventy-two hours after injection. The histologic sections of the vaginal tissue, however, showed very low activity. It became necessary, therefore, to compare the different routes of injection, single versus multiple doses, and to find the best time of killing after injection. The results of this study can be seen in Table VI. The total dose of 0.15 gamma International Standard in oil was given in a single dose or in equally divided doses for three successive days.

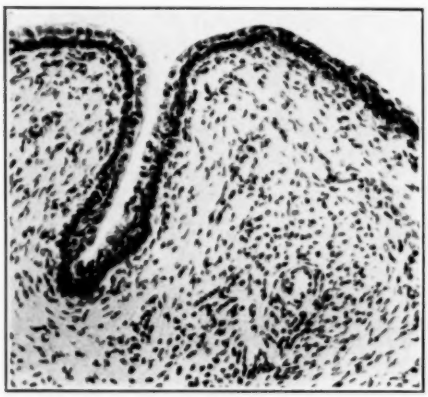


Fig. 6.—Reaction 0.

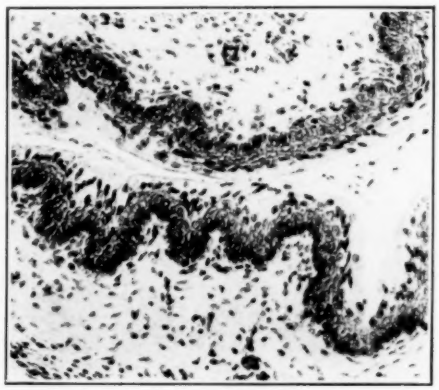


Fig. 7.—Reaction 3.

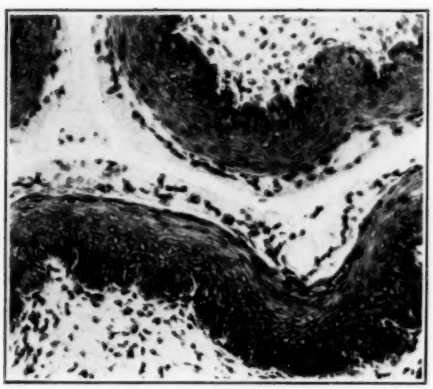


Fig. 8.—Reaction 4.

Fig. 9.—Reaction 5.

On the basis of the results obtained in the above study, the following procedure for testing the activity of estrogenic substances was adopted:

Four or more animals are used for each test. Aqueous solutions are injected subcutaneously in doses of 0.05 c.c. daily at 9:30 A.M., 1:30 P.M., and 4:30 P.M. for three days; the total dose per mouse being 0.45 c.c. The mice are killed twenty hours after the last injection. Oil solutions are injected intramuscularly, 0.05 c.c. per mouse being administered at 1:30 P.M. daily for three days, the total dose given being 0.15 c.c. per mouse. The mice are sacrificed twenty-four hours after the last injection. The vaginal tissue is removed, fixed in Bouin's and embedded in paraffin. Transverse sections are cut from the

middle portion of the vagina, avoiding the regions of the cervix and external orifice. The sections are stained with Harris' hematoxylin-eosin. The reactions described by Fluhmann are used in stating our results. As it is desirable to produce at least a reaction 3, tissue of the castrate type and Reactions 3, 4, and 5 are illustrated in Figs. 6, 7, 8, and 9.

It remained to determine the smallest amount of the International Standard which when injected would produce a pronounced stratification of the vaginal epithelium and the formation of well-developed mucin cells. In order to detect small differences in the dose administered, it was obviously necessary to avoid a maximum reaction, but it seemed desirable to have the majority of the mice show approximately a reaction 4. The results of decreasing doses are shown in Table VII.

TABLE VII

INTERNATIONAL STANDARD (B)

	OIL SOLUTIONS				AQUEOUS SOLUTIONS							
	0.15 GAMMA	0.10 GAMMA	0.08 GAMMA	0.05 GAMMA	0.75 GAMMA	0.3 GAMMA	0.15 GAMMA	0.10 GAMMA	0.09 GAMMA	0.08 GAMMA	0.07 GAMMA	0.06 GAMMA
Reaction 5	5	4	6	1	3	8	6	3	3			
Reaction 4	12	7	5	12		1	12	9	12	4	12	
Reaction 3				12						1	3	4
Reaction 2												
Reaction 1				1								

TABLE VIII

	AQUEOUS SOLUTIONS		OIL SOLUTIONS		
	0.10 GAMMA	0.08 GAMMA	0.10 GAMMA	0.08 GAMMA	0.05 GAMMA
International Standard (B)					
Reaction 5	3		4	6	1
Reaction 4	9	4	7	5	12
Reaction 3		1			2
Reaction 2					
Reaction 1					1
Reaction 0					
International Standard (C)					
Reaction 5					
Reaction 4	3	9	4	7	
Reaction 3		9	1	3	4
Reaction 2					1
Reaction 1					
Reaction 0					
International Standard (D)					
Reaction 5	2				
Reaction 4	1	3		4	
Reaction 3	1	5		5	
Reaction 2					
Reaction 1					
Reaction 0					

The data in Table VII indicate that the desirable amount of the International Standard to be used in assays is about 0.08 gamma.

The results obtained with samples of International Standard, designated C and D, respectively, are shown in comparison with the results obtained with Sample B in Table VII. Samples C and D seemed to produce a weaker reaction than B, but more animals would be required to prove this point.

DISCUSSION

It is often stated that the vaginal smear method should yield results with an error not greater than plus or minus 20 per cent. In our experience, although determinations with groups of twenty rats may often agree within even narrower limits than this, the results may, at any time, without apparent reason, vary by several hundred per cent. Such erratic results may occur with carefully selected groups, injected with the same material, at the same time. We, therefore, consider the vaginal smear method unreliable as a quantitative method unless prohibitive numbers of animals are used.

The mucification method with groups of four animals cannot be depended upon to give results with an error as low as plus or minus 20 per cent, but it certainly is not subject to the wide fluctuations inherent in the vaginal smear method on rats. The condition of the vaginal mucosa is determined by examination of the tissue itself rather than by a smear taken from the lumen of the vagina, thus practically eliminating the personal factor. The animals are uniform in age and period of ovariectomy, and they are used but once so that there can be no question of increasing or diminishing sensitivity or of the influence of differences in the intervals or sizes of preceding doses.

SUMMARY

Results of the examination of 3,000 vaginal smears from rats injected with the International Standard for estrus producing hormone are presented together with a study of the mucification method on 187 mice. The results indicate that:

1. The vaginal smear method is not reliable as a means for comparing products of unknown strength with the International Standard.

2. Results obtained by the mucification method on mice, as used by Fluhmann for the determination of estrin in the blood of women, are more reliable than results obtained by the vaginal smear method.

ACKNOWLEDGMENT

The authors wish to express their gratitude to Frank R. Eldred, Research Director of the Reed and Carnrick Laboratories, under whose supervision this work was done. They are deeply indebted to him for his invaluable advice and criticism.

REFERENCES

1. Memorandum of the International Standard for the Oestrus-producing Hormone. Revision Committee, U. S. Pharm. Circular 369, 1933.
2. Courrier, R., and Raynaud, R.: Experences d'antagonisme humoral ovarien realisies avec l'etalon international de folliculine cristallisee, Comp. rend. Soc. de biol. **115**: 229, 1934.
3. Hinglais, H., and Hinglais, M.: A propos du titrage physiologique de la folliculine cristallisee, Comp. rend. Soc. de biol. **117**: 1005, 1934.
4. Frattini, B.: Dosaggio della follicolina. Unita fisiologiche e unita internazionali, Arch. 1st. biochim. ital. **7**: 1, 1935.

5. Cartland, G. F., Meyer, R. K., Miller, L. C., and Rutz, M. H.: A Comparison of Theelin Prepared from Stallion Urine, Human Urine, and from Theelol, J. Biol. Chem. **109**: 213, 1935.
6. Meyer, R. K., Miller, L. C., and Cartland, G. F.: The Biological Activity of Theelol, J. Biol. Chem. **112**: 597, 1936.
7. Hain, A. M., and Robson, J. M.: Comparative Assay of Oestrone in the Rat and Mouse, J. Pharm. & Exper. Therap. **57**: 337, 1936.
8. D'Amour, F. E., and Gustavson, R. G.: A Biologic Assay of "International Standard" Estrin and of Certain Commercial Preparations, J. Pharm. & Exper. Therap. **57**: 472, 1936.
9. Coward, K. H., and Burn, J. H.: The Variation in the Unit of the Oestrus-producing Hormone, J. Physiol. **63**: 270, 1927.
10. Bulbring, E., and Burn, J. H.: The Estimation of Oestrin and of Male Hormone in Oily Solution, J. Physiol. **85**: 320, 1935.
11. Girard, A.: Adoption d'un systeme international d'unites pour la standardization des preparations de Folliculine, J. Pharmacie **17**: 61, 1933.
12. Fluhmann, C. F.: The Induction of the Pseudo-Pregnancy Vaginal Reaction in Spayed Mice by the Injection of Human Blood, Am. J. Physiol. **95**: 422, 1930.
13. Fluhmann, C. F.: A New Procedure for the Demonstration of Estrin in the Blood of Women, Endocrinology **18**: 705, 1934.
14. Kahnt, L. C., and Doisy, E. A.: The Vaginal Smear Method of Assay of the Ovarian Hormone, Endocrinology **12**: 760, 1928.
15. Rowlands, I. W., and Callow, R. K.: Stability of the International Standard of the Oestrus-producing Hormone in Alcoholic Solution, Biochem. J. **24**: 837, 1935.

PSEUDOAGGLUTINATION OF ERYTHROCYTES BY ALKALI*

R. D. Barnard, M.D., Chicago, Ill.

DURING the course of an investigation on alkali hemolysis it was noted that as a preliminary to hemoglobin liberation, there would be clumping of the erythrocytes in the suspension. Since the appearance of this clumping resembles in some respects the true erythrocytic agglutination brought about by specific agglutinins and might cause confusion in studies related to that phenomenon, the pseudoagglutination produced by alkali was deemed of sufficient importance to report.

When a platinum loopful of blood is placed in a drop of 0.1 per cent sodium cyanide in physiologic saline solution on a microscope slide and the slide is tilted back and forth to mix the drop (as in the performance of the test for blood typing), the erythrocytes will agglutinate. There is in this instance the tendency toward the formation of one large aggregate, whereas in agglutination by specific agglutinin, usually, many small aggregates are formed.

Pseudoagglutination by alkali has been demonstrated in the erythrocytes of the sheep, rabbit, dog, guinea pig and man. No constituent of the serum is responsible since the reaction will take place in washed corpuscles. The rôle of the sodium cyanide appears to be merely that of an alkali since dilute sodium hydroxide in physiologic saline will also evoke the phenomenon. The pH of the cyanide solution is self-buffered at the most favorable point for its demonstration, since it liberates the hemoglobin from the corpuscles very slowly, and hematin formation does not take place.

Pseudoagglutination is apparently due to dissolution of the stroma with the formation of a sticky substance. That the stroma is altered is indicated by the slow hemolysis that goes on progressively at this concentration of alkali. After the hemoglobin has completely escaped into the suspending solution, the ghosts may still be found aggregated.

Pseudoagglutination may be followed macroscopically or microscopically.

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine.

Received for publication, February 15, 1937.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PNEUMONIA, Primary Friedlander, Solomon, S. J. A. M. A. **108**: 12, 1937.

In 27 of 32 cases of primary Friedlander's pneumonia (84 per cent), the involvement was lobar; in the remainder it was bronchopneumonic. As in other pneumonias, certain predisposing factors play a rôle, such as exposure, malnutrition, debility, alcoholism, and antecedent diseases of the upper respiratory tract. The onset is generally acute with chill, fever, cough, and pleuritic pain. Clinically, the patients appear acutely ill, dyspneic, and cyanotic, and there is a fulminating course.

This study reveals certain features which serve to distinguish Friedlander's pneumonia from other forms:

(a) It occurs in the later decades, the average age being 48.7 years.

(b) There is a marked preponderance of males over females, the ratio being 7 to 1.

(c) Hemoptysis is a frequent symptom with the onset (75 per cent).

(d) Herpes is not common (12.5 per cent); jaundice is rather frequent (22 per cent).

(e) In approximately half the cases the frank clinical signs of consolidation are absent when symptomatology suggests it and roentgenograms, fluoroscopy, or necropsy confirms it.

(f) The temperature is less regular and low grade pyrexia is much more common than in pneumococcic pneumonia.

(g) There is frequently involvement of the upper lobe. This occurred alone or in conjunction with lower or middle lobe involvement in 19 cases (59.4 per cent). Involvement of more than one lobe is very frequent. It was noted in 18 cases (56 per cent).

(h) The gross character of the sputum is that of a brick red, homogeneous emulsion of blood and mucus, in contrast to the ropy, rusty sputum of pneumococcic pneumonia. A direct smear of the sputum stained by Gram's method will usually show a predominance of large gram-negative bacilli with a refraction zone around them representing the capsule. Mouse inoculation will invariably confirm the diagnosis. The character of the sputum is pathognomonic and has hitherto not been described in detail.

(i) The Friedlander bacillus is found in pure culture in the sputum, in lung juice, and frequently in the blood culture.

(j) Leucopenia with a "shift to the left" in the Schilling index and monocytosis are frequent.

(k) The mortality rate is very high (97 per cent in this series). Death occurs at an earlier stage in this disease than in pneumococcic pneumonia, the average day of death being between the sixth and seventh days. Death may be attributed to general toxemia and marked pulmonary necrosis, with local vascular changes and pulmonary edema.

(l) Bacteremia is more frequent (73 per cent) but is quantitatively less intense. The mortality rate is apparently little affected by the absence of bacteremia.

(m) Gross abscess formation is the most frequent complication. Empyema and meningitis are less common, and Friedlander's bacillus can be readily cultured from the pus and spinal fluid in these cases.

(n) At necropsy the involved lobes have a characteristic appearance, marked chiefly by some degree of softening and necrosis of the lung with the presence of a characteristic, dirty slimy exudate on the cut surface. Culture of the lung, if the necropsy is performed soon after death, yields the Friedlander bacillus and on microscopic section the organisms can be seen as gram-negative encapsulated rods lying in the cells or free in the alveoli.

When an attempt was made to type the bacillus isolated from the sputum, lung juice, or blood of the patient, a type A organism was found to the exclusion of all other types.

Specific antiserum was used in five cases. Treatment appears to be unsatisfactory, though further trial in a larger series is indicated before any conclusions can be drawn.

The author takes issue with the opinion expressed by Baehr and his coworkers that the Friedlander bacillus is a secondary invader in pneumonia and has cited evidence to refute this view.

PNEUMONIA, Type I, Effects of Very Early Serum Treatment in, Cecil, R. L. J. A. M. A. 108: 687, 1937.

When patients with pneumococcus type I pneumonia are treated very early with homologous serum, the following phenomena are usually observed:

The disease may be completely aborted, the temperature and the pulse and respiration rate dropping to normal within twelve to twenty-four hours after the administration of serum.

There is striking improvement in the patient's general condition as the result of the disappearance of toxemia.

Early serum treatment prevents the spread of infection from one lobe to another and even limits the area of infection in the lobe primarily infected.

Bacteremia is prevented or, if already present, is quickly checked.

The leucocytes return rapidly to normal.

Homologous agglutinins, precipitins, and protective bodies promptly make their appearance in the circulating blood.

Skin tests become positive to the homologous polysaccharide.

The death rate is cut to approximately one-sixth the standard death rate for untreated type I pneumonia.

These conclusions are derived from studies on type I pneumonia, but evidence is rapidly accumulating that they apply with equal validity to several other types as well.

HYDATID (ECHINOCOCCUS) DISEASE in Canada and the United States, Magath, T. B. Am. J. Hyg. 25: 107, 1937.

At least 482 cases of hydatid disease have been recorded in Canada and the United States since the first case seen in 1808.

Ten new cases diagnosed at the Mayo Clinic are added to the cases already reported.

Ninety-five per cent of the cases in which the nationality of the patient is known have occurred among immigrants, and 4 cases have been reported in which the patients were natives born in the United States but who probably acquired the infection in foreign countries.

Three persons most likely acquired the infection in Canada and 19 in the United States, indicating the rarity of the disease among natives who have remained in Canada or the United States.

Infection among males is more frequently seen in Canada and the United States than among females, and children are but rarely infected; only 12 cases in which the patients were children have been reported in more than a century. More than half the total cases occurred among young adults.

The organs most commonly involved are the same as those in countries in which the disease is endemic.

Foreign patients have come chiefly from the countries in which hydatid disease is relatively common and from which relatively many immigrants have come. Iceland, Italy, Germany, Greece, Russia, and Great Britain account for 75 per cent of the cases. Three-fourths of the cases have been reported from New York, Manitoba, California, Massachusetts, Pennsylvania, Illinois, Quebec, Missouri, and Minnesota.

Evidence points to a falling incidence of the disease, as it affects man, in Canada and the United States, and among human beings the curve of incidence flattened out about 1924. There is no evidence that cases are occurring in natives of Canada or the United States with increasing frequency. Some evidence indicates that the incidence among hogs is increasing.

Apparently the usual life history between dog and sheep is not typical in Canada and the United States and this probably explains the almost complete absence of hydatid disease among native human beings. Riley's discovery of a possible natural life history between moose and wolves suggests this or some other similar life history as typical of Canada and the United States, with hogs as accidental secondary hosts and more rarely man.

A native citizen of Canada or the United States, who has never left his country, so rarely has hydatid disease that such an event will occur less often than once in about five years; this is stated on the basis of past experience.

Measures should be studied to prevent the increase of this disease in Canada and the United States, especially keeping in mind Riley's important observations.

BLOOD GROUPING, a Simple Technic for Determination From Small Amounts of Defibrinated Blood, Hoxworth, P., and Ames, Z. J. A. M. A. **108:** 1234, 1937.

Approximately ten drops of blood is obtained by finger puncture and defibrinated in a Wassermann tube by whipping with a wooden applicator for five minutes. This quantity is sufficient for grouping and several compatibility tests. Separation of cells and serum is not desirable.

Grouping: Large drops of high titered test serums anti-B and anti-A are placed on the left and right ends respectively of a glass slide. Defibrinated blood is added to each drop of serum and thoroughly mixed with the aid of a platinum loop. The slide is tilted back and forth over a white background in bright light and observed macroscopically for clumping. Agglutination is striking and, if serum of grade I is used, will be complete in less than one minute. When no agglutination is seen after one minute on either side, the slide is placed under a Petri dish with wet blotting paper and observed again fifteen minutes later as a precaution against weak agglutinogens.

Matching: A 1:1 or 50 per cent suspension of the recipient's defibrinated blood is prepared in the stem of a white blood cell counting pipette by drawing blood up to the 0.5 mark and then filling to the 1.0 mark with physiologic solution of sodium chloride. The blood is diluted 1:1 with saline solution because agglutination cannot be differentiated microscopically in any greater concentration and because dilution inhibits undesirable rouleau formation. The diluted blood is deposited on the left end of a glass slide and mixed by means of a jet of air blown from the pipette. An identically prepared suspension of donor's defibrinated blood is placed on the right end of the same slide, and one-fifth of this drop (two divisions of the pipette) is transferred to the drop of diluted recipient's blood. This 1:5 ratio is based on the assumption that 500 c.c. is to be transfused to a patient whose blood volume is depleted 50 per cent or approximately to 2,500 c.c. It provides a wide margin of safety in transfusions from a "universal" donor, as dangerously potent agglutinins will not be completely absorbed in this concentration without manifest agglutination. The 1:5 mixture of donor's and recipient's blood is then stirred with a platinum loop or glass rod, and the slide is placed under an inverted Petri dish with a piece of wet blotting paper. After fifteen minutes the mixture is again agitated and observed microscopically under low power for agglutination.

Care must be taken not to confuse rouleau formation with agglutination. The former can always be broken up by stirring, while the latter will be intensified. Agglutination will be striking if a grouping error has been made but a little practice is required to estimate the degree of agglutination of the recipient's cells by a "universal" donor's agglutinins, in the presence of nonagglutinable cells forming rouleaux. Unless the agglutination is so definite and conspicuous that there is no question of confusion with rouleau formation, the blood may be pronounced compatible.

ANEMIA, PERNICIOUS, The Sternal Marrow in, Damashek, W., and Valentine, E. H. Arch. Path. **23:** 159, 1937.

Biopsies of the sternal marrow were made in twenty cases of pernicious anemia at various stages both before and after treatment with antianemic substances. Both sections and smears made directly from the marrow were studied. For exact cellular differentiation and for differential counts, the smears were found to be much more satisfactory than the sections.

The changes in the marrow were correlated with the status of the peripheral blood. In the cases in which treatment had been given the findings varied according to the time of the biopsy in relation to the reticulocyte peak. Before the reticulocyte peak, the marrow showed evidence of marked activity with many mitotic figures. Within a few days after the reticulocyte peak there was almost complete disappearance of megaloblastic cells, accompanied by a concomitant increase in the numbers of cells of the normoblastic series. In two cases in which the condition relapsed while under active parenteral treatment with liver, intense megaloblastic hyperplasia was present.

An associated hyperplasia of the granulocytic cells was observed together with the presence of many bizarre forms of metamyelocytes and large numbers of typical lymphocytes.

On the basis of the aforementioned and correlated observations, it may be stated that there are two types of erythroblastic hyperplasia: (1) the normoblastic (occurring in iron deficient states, polycythemia, congenital hemolytic icterus, etc.) and (2) the megaloblastic (occurring in "liver deficient" states, or pernicious anemia). The most primitive red cell present in both these conditions is the erythrogone.

In the state of "liver deficiency" (pernicious anemia), erythropoiesis becomes arrested at the erythrogone level, and whatever maturation takes place is in the direction of the abnormal megaloblast, the last stage of which is the macrocyte. Thus, both arrest of maturation and maldevelopment are present. This is also exemplified in the condition of the granulocytic cells, which often show monstrosities of cellular development, the end-results of which are probably the many-lobed polymorphonuclear cells of the peripheral blood.

The changes in (1) the erythroblastic cells, (2) the granulocytes, and (3) the megakaryocytes suggest that pernicious anemia is a disease affecting the entire marrow. Other studies have shown that this is dependent on a deficiency in what is conveniently called a "liver substance." The striking and almost incredibly rapid effects of liver extract on the marrow are seen in (1) the disappearance of erythrogones and megaloblasts and their conversion to normoblasts, (2) the disappearance of abnormally shaped granulocytes, (3) the disappearance of lymphocytes, and (4) the increase in the number of megakaryocytes. Liver extract appears to supply a material which is necessary for the normal development of all the cells in the marrow. In the absence of this substance arrest of maturation and maldevelopment take place. This is reflected in the peripheral blood as anemia of the macrocytic type, leukopenia with the presence of many-lobed (pernicious anemia) neutrophils and thrombocytopenia.

NEOPLASMS, The Identification of Tumor Cells in Sediments of Serous Effusions, Foot, N. C. Am. J. Path. **13**: 1, 1937.

Summarizing the findings of a brief investigation, the author suggests that we should revise the criteria for reporting "tumor cells present" in a given fluid in somewhat the following manner:

Zemansky's first criterion, the presence of fragments of tumor with the cells arranged in acini or papillae about a stroma that is definitely fibrovascular, stands uncontroverted.

A nucleolar-nuclear ratio falling above 0.25 is of undoubted value; one of 0.30 or more practically pathognomonic of the presence of tumor.

Mesothelial pleural, pericardial, and peritoneal covering cells present the chief obstacle in the way of successful diagnosis as they are readily confused with tumor cells on account of their large size and prominent nucleus. When they are measured the dimensions are found to be quite uniform and regular; tumor cells, on the other hand, show a high n/N ratio and a wide variation in measurements.

Multinucleation is of no diagnostic value and cell clumping is almost as worthless. Mitosis occurs in both positive and negative sediments, but monster, or abnormal, mitoses are found only in tumor cells.

The occurrence of erythrocytes and fibrin is of little diagnostic value.

BLOOD SEDIMENTATION RATE, Influence of External Temperature on, Rosenthal, D. B. M. J. Australia **1**: 172, 1937.

The sedimentation rate of blood is greatly affected by the external temperature; the higher the temperature, the faster the rate.

The change in rate is roughly proportional to the change in temperature.

The change in rate with alteration of temperature is attributable, in part at least, to the change in viscosity of the blood plasma.

In the absence of any thermometric standard, comparison of tests performed at different times on blood from the same source provides no reliable information.

It is suggested that the reading of the "sedimentation index" should be accompanied by that of the room temperature and that of the cell volume for the specimen.

TISSUE: A Cellophane Embedding Box, Ferguson, F. F. Stain Techn. **12**: 71, 1937.

A new paraffin embedding technique involves the use of embedding boxes made of No. 450 M. T. cellophane instead of paper boxes. They are made by cutting and folding the material just as paper boxes are made. This new method has these advantages:

1. The fixed material is dehydrated, cleared, and paraffin is added, without leaving the cellophane box.

2. The transparent box allows the smallest material to be observed easily under the dissecting microscope.

3. The cellophane separates from the paraffin cleanly.

Pipettes with annealed tips are recommended for handling small material when using this technique.

TANNIC ACID, Compound Solution of, Fantus, B., and Dyniewicz, H. A. J. A. M. A. **109**: 200, 1937.

The authors present the formula below for the treatment of burns as having the following advantages:

It is endowed with good keeping qualities.

It produces a denser coagulum.

It has bacteriostatic action, which is absent in the plain tannic acid solution.

COMPOUND SOLUTION OF TANNIC ACID

Potassium chloride	0.42 gm.
Calcium chloride	0.84 gm.
Salicylic acid	1.00 gm.
Sodium chloride	10.50 gm.
Tannic acid	100.00 gm.
Distilled water	1,000.00 c.c.

Mix and permit to stand with occasional agitation until dissolved and filter, if required to dispense a clear solution.

TULAREMIA, The Cause of Death in, Foshay, L. Arch. Int. Med. **60**: 22, 1937.

The chief cause of death attributable to tularemia alone is septicemia due to *Bact. tularense*. Pneumonic lesions are present in about half the fatal cases of tularemia. Most cases of pneumonia that are associated with fatalities derive from the septicemia. Pneumonia that originates from the primary bacteremia does not become associated with fatalities unless septicemia supervenes. At least 70 per cent of the cases of tularemia pneumonia do not result in death. In a small number of patients, probably much smaller than the proportion in this series indicates, the primary bacteremia is septicemia from the onset and causes a rapidly fatal termination in from four to ten days. These unfortunate persons have apparently no natural resistance to the infection. The septicemia which causes most fatalities

originates from a second invasion of the blood stream. Tularemic sepsis, with its consequent miliary focal necroses, may be generalized or may be limited to either the systemic or the pulmonary circulation. In this respect it is analogous to miliary tuberculosis. This analogy may be extended to the modes of origin of septicemia.

The mortality rate of the typhoid type of tularemia is four times that of the other clinical types. The incidence of pneumonia for this type is four times the average incidences in the three other types and is three times larger than the incidence of pneumonia in tularemia in general.

Tularemia is especially dangerous to persons with pre-existing coronary heart disease. Death may occur in such cases from coronary occlusion or acute myocardial failure during the initial acute phase or during early or late convalescence. The surviving patient may suffer attacks of angina pectoris, coronary occlusion and heart block for months or years after recovery from tularemia.

Persisting, progressive tularemic lesions which ultimately involve important structures are infrequent causes of death late in the course of the disease.

The third week of infection is the most dangerous period, and most deaths occur on the sixteenth day of illness. The disease is characterized by severe toxemia, which appears, of itself, seldom or never to cause death among patients who were healthy at the time tularemia was contracted. Persons over fifty years of age tolerate the infection less well than younger persons.

It is estimated that 4 of every 5 deaths from tularemia could have been prevented by the early administration of serum therapy.

INFLAMMATION, The Mechanism of, Menkin, V. Arch. Path. 24: 65, 1937.

The author summarizes the salient features of inflammation as follows:

The initial increase in plasma filtration occurring in inflammatory conditions seems to be primarily referable to an increase in the intracapillary pressure and in the permeability of the minute vessels. There is evidence that the increase in permeability of the endothelial wall is referable to a liberation from injured tissue of a diffusible crystalline substance other than histamine. This factor can be isolated from exudates and recovered in detectable quantities. It is capable of inducing pronounced seepage through the capillary wall. Passage of plasma proteins, including fibrinogen, into the injured part is thus favored at a very early stage of the reaction. Prompt coagulation of the plasma and formation of obstructive thrombi in the afferent lymphatics circumscribe the area of injury, inhibiting the dissemination of the irritant. Thus the primary mechanism of fixation inflammation, which under some circumstances may be re-enforced by various secondary factors, plays a definite rôle in immunity. The prompt early walling off of the irritant allows the leucocytes time to collect at the site of inflammation for phagocytosis. The rapidity and intensity of the fixation are largely a function of the irritant. Irritants that cause necrosis induce an extremely prompt local reaction inhibiting their spread. The reverse is true of weak irritants. Observations on the behavior of various pyogenic micro-organisms indicate the rôle of the inflammatory reaction in regulating the rapidity of invasiveness and therefore the significance of this reaction in grading the immunity or resistance of the host. In brief, the dissemination of micro-organisms seems, at least during a given interval, to be inversely related to the intensity of the local injury which they cause.

There is definite evidence that the migration of polymorphonuclear leucocytes in inflammation is related to the liberation of the nitrogenous crystalline substance which can be recovered from exudates and which induces initially an increase in the permeability of capillaries. The available evidences indicate that this substance probably belongs to the group of polypeptides. It possibly represents the result of an interference with normal protein catabolism in an area of injury. The tentative name "leukotaxine" is proposed for this active substance which is per se capable of rapidly reproducing the basic sequences of the inflammatory reaction.

Finally the disturbance in local circulation which doubtless occurs at the site of inflammation as a result of enhanced capillary filtration, lymphatic blockade and subsequent capillary thrombosis tends, by shunting off the injured area, to favor development of local

acidosis. This results from a disturbance of the local intermediary carbohydrate metabolism, as evidenced by increased glycolysis, and from a depletion of the alkali reserve. At a given time the prevailing concentration of hydrogen ions in the exudate seems to play a significant rôle in the determination of the cytologic picture. Observations indicate that the marked fall in the pH is associated with an overwhelming lethal effect on all types of leucocytes, and that frank suppuration is the result.

SPIROCHETA PALLIDA, New Stain for, de Castro Cerqueira, A. G. Brasil-med. 50: 969, 1936.

The author describes a technic which is a modification of Holland's method. The smears are obtained from skin and mucous membrane lesions or by gland puncture. The smear is fixed for fifteen to thirty seconds in absolute or 90 degree alcohol. The alcohol is poured off and, without drying, a few drops of a hydro-alcohol solution of tannin-acetic acid is poured over the smear. This solution is a modification of Holland's preparation as follows: Chemically pure tannin, 5 gm.; glacial acetic acid, 4 c.c.; absolute or 90 degree alcohol, 4 c.c.; distilled water, 50 c.c.

The slide is then heated over a low flame until vapor begins to pass off. It is then washed rapidly with tap or filtered water. The use of distilled water is not necessary. It is stained with Ziehl's phenolized fuchsin heated until it begins to vaporize. It is then washed rapidly in tap water and dried.

During the last heating the slide should be inclined slightly so that any excess of the stain will flow off after having impregnated the smear. The slide should be held at some distance from the flame in heating; thirty to sixty seconds are sufficient to give clear and accurate pictures. The slides can be kept for months. The spirochetae are stained a pale pinkish violet. Spirilla and other bacteria are stained more intensely.

This method gives very clear microscopic pictures.

HYPERGLOBULINEMIA, Formol-Gel Reaction as a Preliminary Test for, Wise, C. R., and Gutman, A. B. Am. J. M. Sc. **194**: 263, 1937.

The formol-gel test was performed in 113 serums of which the content of total protein and of the several protein fractions was determined. The results of the formol-gel reaction were quantitated according to a clinically practical schema in which changes in gelation and opacity were recorded at arbitrarily selected periods of five minutes, two hours, and twenty-four hours. The rapidity and intensity of changes in gelation and opacity were correlated with the content of total protein, globulin and the several globulin fractions.

All but one of 24 serums with total proteins of 8.5 per cent or more and all but one of 35 with globulin of 4 per cent or more showed changes in viscosity and opacity within twenty-four hours. Conversely, all but one of 52 serums with globulin levels below 3.4 per cent were negative after twenty-four hours. Serums containing 3.4 to 4 per cent globulin could not be detected consistently by means of the formol-gel test. Sera with total proteins less than 8 per cent gave positive results if hyperglobulinemia was present.

Of 35 serums with globulin contents of 4 per cent or over, 30 became positive within two hours. Most positive reactions obtained in serums with globulin contents between 3.4 per cent and 4 per cent, on the other hand, required more than two hours to develop. Formation of an opaque or semiopaque-gel within five minutes occurred only in serums containing well over 5 per cent globulin.

The data suggest that the formol-gel reaction is consistent enough to serve as a convenient preliminary test for the detection of hyperglobulinemia of diverse origin. Possible applications of the test are considered. The formol-gel reaction is a diagnostic aid only so far as it is positive in diseases presenting hyperglobulinemia.

The results are in accord with the view that the formation of an opaque gel is due to qualitative as well as quantitative changes in the serum globulin fraction. The altered serum globulins responsible for opaque gell formation do not have solubility characteristics with respect to sodium sulphate which permit of their classification as euglobulin or pseudoglobulin, as determined by Howe's method.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Little Things of Life*

"IN THIS book the story of the vitamins, the mineral elements and the ductless glands in relation to nutrition and the health of man is told in non-technical language." Since these substances are present in such small amounts, although of great physiological importance, the author has selected the title, "The Little Things of Life." Not only the nutritional elements and hormones but also the diseases due to a defect or excess supply of these substances are discussed.

This book is very interestingly written and is a valuable addition to the publications in this field. It is intended primarily for the layman but should be most profitable reading for the physician also.

R. L. H.

The Fundamentals of Electrocardiographic Interpretation†

THIS book may be recommended without reserve, not only to those now engaged in electrocardiography, but especially to those desirous of informing themselves upon the utilization of this procedure in the study of disease.

As is said in the foreword by H. B. Williams, Professor of Physiology in the College of Physicians and Surgeons: "Any physician who will study this work with care should acquire ability to interpret the simpler records without difficulty."

In view of all too common tendency to confer at times upon various laboratory procedures an unwarranted importance, and to base unwarranted inferences upon them, Dr. Carter has wisely emphasized that the electrocardiogram is an adjunct to clinical methods which it must not be used to supplant.

The volume abounds with illustrative electrocardiograms wisely chosen, conservatively interpreted, and well reproduced.

There is a full bibliography covering the years 1916-1935, and an excellent index.

An interesting innovation is a note to the reader that lantern slides of the illustrations, complete or in groups, are available.

It may be predicted with confidence that this book will enjoy a wide circulation as an outstanding text upon electrocardiography.

---

*The Little Things of Life. By Barnett Sure, Ph.D., Professor of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark. Cloth, 340 pages. D. Appleton-Century Co., New York and London, 1937.

†The Fundamentals of Electrocardiographic Interpretation. By J. Bailey Carter, Clinical Instructor, Dept. of Medicine, Rush Medical College, 326 pages, 250 figures, $4.50. Charles C. Thomas, Springfield, Ill.